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Bioremediation of Marine Oil Pollution

by

Dr. David L. Gutnick

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16. Abstract

This report presents an assessment of the scientific and technological developments in the area of bioremediation and biodegradation of marine oil pollution. A number of allied technologies are also considered. Many of the topics discussed are presented in a summary of a Workshop on Bioremediation of Marine Oil Pollution sponsored by TDC. The summary includes an overview of the formal presentations as well as the results of the working groups.

A number of programs in bioremediation are recommended to the management of TDC. These programs and a coordinated framework in which to implement them are outlined. The projects are designed to utilize Canadian expertise to develop a number of transport-related biotechnologies.

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16. Résumé

Ce rapport évalue les avancées scientifiques et techniques dans le domaine de la biodégradation tant naturelle qu'artificielle et celui des procédés connexes de lutte contre la pollution des mers par les hydrocarbures. Nombre des sujets abordés figurent dans le compte rendu d'un atelier organisé par le CDT sur ce même thème, où sont résumés les propos des conférenciers ainsi que le résultat de la réflexion des groupes de travail qui se sont formés.

Un certain nombre de projets de recherche ainsi que le cadre pour leur coordination et leur mise en oeuvre sont proposés et définis. Mettant à profit les compétences canadiennes existantes, ces projets visent le développement de biotechnologies applicables à la solution de problèmes liés aux transports.

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SUMMARY

Bioremediation of oil pollution is one of the spill clean-up technologies to receive great attention in recent years. However, even after the enormous efforts to demonstrate the efficacy of the technology in the *Exxon-Valdez* spill, bioremediation still remains somewhat controversial and its effectiveness has been called into question. Nevertheless, many feel that this approach represents one of the most promising of the emerging technologies.

In this context, the author of this report was invited to the Transportation Development Centre (TDC) as a Visiting Expert under its Visiting Experts Program. He was asked to prepare a comprehensive assessment of the potential application of bioremediation and biodegradation measures to combat the effects of marine oil spills, to recommend avenues of research to be pursued, and to identify the available research and development capabilities in Canada. As part of his work he was asked to plan, organize, and chair a workshop on bioremediation of marine oil pollution.

This report presents the results and conclusions of these efforts.

The basic technology in bioremediation involves the addition of fertilizers to an oil spill to enhance the natural process of oil biodegradation. Bioremediation can be applied to open systems such as beach or land spills, or it can be applied in closed, more controllable environments such as oil storage containers, specially constructed or modified bioreactors, and cargo tanks. Bioremediation has yet to be successfully demonstrated in the open sea. The major advantage of using closed environments is the opportunity to control the physical and nutritional parameters to optimize the rate of biodegradation. Moreover, seeding with more suitably adapted micro-organisms may be more applicable in closed environments.

By and large the author found Canadian expertise to be at a par with the best-known groups in the world. Similar high levels of accomplishment were found for a number of allied technologies. Given this situation, the author has recommended a number of programs and has outlined a framework in which to develop these efforts.

It is recommended that initially TDC get involved in short-term manageable projects in which the expertise of TDC personnel in ship design and operations, marine engineering, materials, and maintenance can be brought to bear on a specific area. Three such short-term projects currently not being examined elsewhere would involve preliminary laboratory and field work designed to test the feasibility of the following:

1. The use of a barge as a mobile bioreactor for the treatment of off-loaded oily waste products;

2. The use of *in-situ* bioremediation to carry out extensive cleaning, de-gassing, and sludge remediation on board an oil tanker;

3. The use of a barge as a mobile bioreactor and facility for the bioremediation of bilges.

Projects that would be preliminary in nature include:

4. Bioremediation of deicing fluids, primarily for airports;

5. The determination of the biological basis of corrosion on the outside hulls of ships.

The last two projects are more experimental, but their effects on transport-related environmental problems are well recognized. The first project, converting a barge to a mobile bioreactor, having already met with some enthusiasm from senior officials in the Coast Guard because of certain waste disposal problems in the Arctic, should definitely receive the highest priority. The author already has experience in the tanker project, while the bilge remediation project may require an examination of specific bilge materials, which may contain waste materials other than oil. All these projects have defined benchmarks and milestones and can be designed around closely coordinated preliminary studies, including work on the barge or the tanker itself.

It is recommended that TDC consider a phased entrance into the area of environmental and transport-related biotechnology. Phase I includes the execution and evaluation of the short-term project(s) presented above. Phase II would require a closer evaluation and in-depth analysis of the TDC experience in the management of such projects with a view to deciding how best to proceed. Should the decision in Phase II be positive, it is recommended that nothing proceed before the taking on of at least one additional staff member, with a background in biology (preferably biochemical engineering), who could move the program into the longer-term R&D projects. Such projects would probably consider design and retrofitting of barges for various bioremediation activities.

These projects are designed to utilize Canadian expertise to develop a number of transport-related technologies and applications. It is the author's opinion that the success of any of these programs will thrust Canada to the forefront of technological development in this area.

SOMMAIRE

Depuis quelques années, la biodégradation artificielle suscite un intérêt croissant, car c'est l'une des technologies émergentes les plus prometteuses pour le nettoyage des déversements accidentels d'hydrocarbures. Cependant, la polémique fait rage autour de cette technologie. On est allé jusqu'à remettre son efficacité en cause, même après l'effort gigantesque de démonstration de cette efficacité durant la campagne de nettoyage qui a fait suite au naufrage de l'*Exxon-Valdez*.

C'est dans un tel contexte que l'auteur du présent rapport a été invité par le Centre de développement des transports (CDT) au titre de son programme d'experts invités. Son mandat : faire une évaluation exhaustive des possibilités offertes par les techniques de biodégradation tant naturelle qu'artificielle dans la lutte contre les déversements accidentels d'hydrocarbures en mer, préconiser les voies de recherche à explorer et identifier les compétences existant au Canada en matière de recherche et développement dans ce domaine. Dans cette lancée, il a du préparer, organiser et présider un atelier sur le thème de la dépollution par des procédés biologiques.

Le présent rapport rend compte des résultats obtenus à la suite des recherches effectuées par l'auteur.

La biodégradation artificielle consiste essentiellement à répandre des matières nutritives sur la nappe d'hydrocarbures de manière à activer et accélérer leur biodégradation, qui est un phénomène naturel. Cette technique peut être utilisée aussi bien dans un environnement ouvert, une plage ou un sol pollués, par exemple, que dans un environnement fermé : réservoir de stockage d'hydrocarbures, bioréacteur modifié ou conçu exprès ou soutes d'un navire-citerne, qui sont des environnements sur lesquels il est plus facile d'agir. Mais dans le cas des déversements en mer, son efficacité n'a pas encore été pleinement démontrée. Les environnements fermés offrent le grand avantage qu'ils permettent de maîtriser les paramètres physiques et biologiques en jeu de manière à optimiser le processus de biodégradation. De plus, il est possible dans ces cas de procéder par ensemencement, ce qui consiste à introduire des micro-organismes particulièrement adaptés à l'environnement visé.

L'auteur estime que, dans l'ensemble, le Canada tient bien sa place parmi les nations les plus compétentes au monde, non seulement dans ce domaine particulier, mais aussi dans des domaines connexes. Il a donc recommandé la mise sur pied d'un certain nombre de programmes, dont il a défini grosso modo le cadre de la mise en oeuvre.

Le CDT, a-t-il préconisé, devrait commencer par lancer des projets de R&D à court terme et faciles à gérer, pour lesquels il sera possible de faire appel aux compétences que le CDT

possède en matière d'architecture et de mécanique navales, d'exploitation maritime, de matériaux et de maintenance et de les appliquer à des cas particuliers. Il a proposé trois de ces projets, jamais encore envisagés et qui nécessiteraient des recherches préliminaires en laboratoire et des expérimentations in situ conçues pour vérifier la possibilité :

1. d'utiliser une barge comme bioréacteur mobile dans lequel seront traités les déchets d'hydrocarbures provenant de navires;

2. d'utiliser les techniques de biodégradation artificielle dans un navire-citerne pour qu'elles y opèrent des actions de nettoyage, de dégazage et d'élimination des boues à grande échelle;

3. d'utiliser une barge comme bioréacteur mobile pour le nettoyage des eaux de cale par des procédés de biodégradation artificielle.

De plus, les deux projets suivants, encore à l'étape préliminaire, sont également proposés :

4. nettoyage par la biodégradation artificielle des agents de dégivrage, surtout en milieu aéroportuaire;

5. détermination des causes de la corrosion biologique s'attaquant aux coques des navires depuis l'extérieur.

Ces deux derniers sont encore au stade des expérimentations, mais leur effet sur divers problèmes de pollution associés aux transports maritimes n'en est pas moins reconnu. Le premier projet, qui consiste à transformer une barge en bioréacteur mobile, a déjà suscité un certain intérêt de la part de la Garde côtière canadienne qui cherche une solution à certains problèmes d'élimination des déchets dans l'Arctique; il devrait donc constituer un projet hautement prioritaire. L'auteur a déjà travaillé dans le domaine visé par le deuxième projet, celui du nettoyage à bord d'un navire-citerne. Quant au troisième projet, celui du nettoyage des eaux de cale, il pourra nécessiter une étude sur le contenu éventuel de certaines d'entre elles qui pourraient renfermer des déchets autres que des hydrocarbures. Tous ces projets, pour lesquels des points repères et des dates jalons ont été définis, pourront être intégrés à l'étape préliminaire de certaines études, comme d'une barge, ou d'un navire-citerne.

L'auteur recommande que le CDT ne fasse son entrée dans le domaine des biotechnologies environnementales et liées aux transports que par étapes. Après la réalisation et l'évaluation des projets à court terme déjà cités, le CDT ne devra poursuivre qu'à la lumière d'une analyse fine et en profondeur des résultats obtenus en matière de gestion.

Et même si ces résultats s'avéraient positifs, il faudra attendre que soit engagée au moins une personne ayant eu une formation en biologie (préférablement en biochimie), apte à faire le joint avec les programmes de recherche à long terme. Il est envisageable que ceux-ci puissent intéresser la réalisation de barges soit conçues exprès, soit aménagées pour y mener diverses opérations de biodégradation artificielle.

Les projets en question, mettant à profit les compétences canadiennes existantes, déboucheront sur le développement de biotechnologies applicables à la solution de problèmes liés aux transports qui, selon l'auteur du rapport, placeront le Canada à l'avant-garde des pays oeuvrant dans ce domaine.

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1. INTRODUCTION

Recent oil spills in Alaska, the Gulf of Mexico, Italy, and the State of Washington, as well as the intentional dumping of over 2 million tons of oil in the Persian Gulf, have galvanized public concern throughout the world, leading to serious international efforts to bring such major discharges under control. According to a recent article in *Scientific American* (1), tanker accidents currently contribute about 5 percent of the estimated 2.3 million tons of petroleum hydrocarbons entering the seas each year. As one of the most intensively studied oil spills, the *Exxon Valdez* incident has catalyzed a major evaluation (and re-evaluation) of clean-up technology. For example, in the U.S. a clean-up fund of $1 billion per spill has been established. In addition, the oil industry in the U.S. has committed $900 million to a new Marine Spill Response Corporation, which plans to spend between $30 and $35 million on research and development of related technology over the next five years. The Exxon Corporation itself spent over $2.5 billion on clean-up in Alaska (1).

In Canada, much concern has been expressed recently about the ability to respond to significant oil spills, particularly in the Brander-Smith Report on *Protecting Our Waters* (2), the Anderson Report to the Premier of British Columbia on *Oil Transportation and Oil Spills* (3), and the Canadian Petroleum and Independent Petroleum Associations' Report on *Oil Spill Preparedness of the Upstream Petroleum Industry* (4). As an indication of the magnitude of the concern, the cost of implementing the 107 recommendations of the Brander-Smith report is estimated at $1.5 billion. Moreover, in the total field of environmental protection, the Green Plan calls for the Canadian Government to spend approximately $3.5 billion over the next ten years. Some $100 million of this money has been allotted to Transport Canada, Environment Canada, and Fisheries and Oceans Canada, specifically for their response to marine environmental emergencies.

Bioremediation of oil pollution is one of the spill clean-up technologies to receive great attention in recent years. This process involves the use of micro-organisms to transform and degrade harmful substances into non-toxic compounds. At a cost of some $40 million, the attempt at beach clean-up in Valdez using bioremediation was the largest experiment of its kind ever performed in the field (5). However, even after the enormous efforts to demonstrate the efficacy of the technology in the *Exxon-Valdez* spill, bioremediation still remains somewhat controversial and its effectiveness has been called into question. Nevertheless, many feel that this approach represents one of the most promising of the emerging technologies.

In this context, the author of this report was invited to the Transportation Development Centre (TDC) as a Visiting Expert under the Visiting Experts Program. The objectives of the project were:

- to prepare an assessment of the state of the art of bioremediation of oil pollution in Canada;
- after formulating an agenda, to organize and chair a nationwide Workshop on Bioremediation;
- using the information gathered in the above activities, to identify outstanding issues and potential research and development programs in bioremediation of oil pollution. Given the appropriate structure, as well as adequate funding and management personnel, these programs could provide the framework for TDC involvement in this area.

The information, conclusions, and recommendations presented in the report stem from:

- numerous meetings and in-depth conversations conducted throughout Canada with key players in government, industry, and academia;
- the author's own extensive experience in all aspects of petroleum microbiology;
- numerous reports, technical papers, and operations manuals;
- the presentations and recommendations emerging from the Workshop on Bioremediation of Marine Oil Pollution organized by the author and held in Montreal on 3 October 1991.

2. TECHNICAL REVIEW OF BIOREMEDIATION

2.1 The science[1]

2.1.1 What is bioremediation?

There are very few organic (carbon-containing) compounds in nature that cannot be degraded by one or more micro-organisms, such as bacteria, fungi, or yeast. In this way organic molecules serve as sources of carbon and energy for the particular micro-organism. Frequently, this degradation process, termed *bio*degradation, brings about the complete breakdown of the carbon compound to carbon dioxide and water. A practical application of such a system is a municipal sewage treatment plant in which natural organic waste material present in water is completely combusted to CO_2. In other circumstances the organic compound may be only partially degraded. If the organic compound(s) is toxic or aesthetically undesirable, this partial degradation frequently results in its conversion (or *biotransformation*) into less toxic or non-toxic compounds. In addition to carbon and water, micro-organisms require additional nutrients for growth, such as sources of nitrogen, phosphate, trace metals, and sometimes oxygen, all of which may be present in very small amounts in certain natural environments. Consequently, the growth of pollution-degrading organisms in nature may be limited by nutrient availability as well as by a number of other factors. The process of bioremediation involves harnessing the natural process of biodegradation of particular contaminants by promoting the growth of micro-organisms that can convert them to non-toxic by-products.

2.1.2 The enrichment culture

The microbial world contains thousands of different varieties and species. Because of the constantly changing environments, the relative numbers of organisms within a natural environment will be in a dynamic state of flux. These environments are frequently depleted in essential nutrients (termed *oligotrophic*) and the various strains will generally exist in a state of competition. When the environment is supplemented with a particular carbon source, i.e., some form of pollution, conditions will have been established that confer a specific advantage on those organisms with the ability to utilize the specific contaminant as a growth substrate. As a result, the environment is said to become relatively enriched with pollutant-degrading micro-organisms. Bioremediation involves supplementing the environment with nutrients and conditions favouring the growth of the specific pollution-

[1] *For further background the reader is referred to an excellent brochure published by the U.S. Environmental Protection Agency (EPA) entitled* Understanding Bioremediation, A Guide Book For Citizens *(17).*

degrading organisms over other microbes naturally occurring in the environment. It has been observed that the initial numbers of oil-degrading micro-organisms within an environment are highest in those areas that have been subject to hydrocarbon pollution due to natural seepage, major spills and other disasters, or chronic forms of oil pollution (6). For this reason, most approaches to bioremediation rely on those populations already present in the polluted environment. Some groups have advocated the addition of more suitable microbial populations (seeding) to treat oil pollution. Seeding may in fact prove effective in environments such as the open sea, in which the natural microbial population may be quite low, although this remains to be demonstrated.

2.1.3 Oil as carbon source

Three major features of fossil oil make it a particularly interesting material for bioremediation: its chemical heterogeneity – crude oil consists of over 2000 different materials; its water insolubility and physical characteristics; and its chemical structure – since oil consists of hydrocarbons, most of which are completely devoid of oxygen, almost all significant biodegradation will require the availability of air (oxygen) as an essential nutrient.

Chemical heterogeneity

Crude oil contains virtually thousands of different compounds that can be grouped roughly according to their relative solubility and chemical structure. Oil may contain anywhere from 30 to 60 percent alkanes, straight chain molecules ranging in size from 10 to about 22 carbon atoms in length. Most oil degrading micro-organisms seem to be able to utilize this fraction of the oil. Another major component of fossil fuels is the simple aromatic fraction (benzene, toluene, xylene, etc.). As its name implies, this fraction of oil is highly volatile and, in most oil spills, it may evaporate before any clean-up effort can be mounted. Interestingly, an organism capable of both aromatic and alkane degradation has yet to be isolated, indicating a reasonable degree of specificity among the various microbes. In addition, organisms have been isolated from nature on the basis of their ability to utilize either naphthenics (more complex aromatics such as naphthalene derivatives) or paraffinic hydrocarbons (waxes). Since the degradation of the different fractions is relatively specific, different micro-organisms are required to catalyze their breakdown. The pathways are relatively independent, so that maximum oil degradation is effected through the activity of several different strains operating cooperatively (7, 8). Such a mixed population of oil-degrading bacteria has been termed a *consortium*. Bioremediation of mixed substrates, such as crude or even refined fossil fuel oils, will always involve the activities of microbial consortia. Since components of petroleum degrade at different rates,

to speak in terms of an overall rate for oil degradation is difficult and misleading. Rates are typically highest for the saturated alkanes, followed by the light aromatics, with the higher molecular weight aromatics, asphaltenes, and resins exhibiting extremely low rates of degradation (6).

Another fraction consists largely of complex Poly Aromatic Hydrocarbons (PAHs). These materials are quite difficult to analyse chemically, and consequently monitoring their biodegradation is extremely difficult. The problem is particularly acute in terrestrial environments, where precise determination of the PAH levels in a polluted soil is frequently difficult. Some evidence has recently been presented demonstrating that PAH biodegradation may be carried out to a limited extent under anaerobic conditions (9,10,11).

The last fraction of crude oil is the asphaltenic fraction. The asphaltenes represent complex networks of polymerized cyclic materials, which are the main constituents of tar. These molecules are particularly resistant to microbial degradation, and although some researchers have reported partial degradation of asphaltenes (12), their concentration within the oil fraction actually increases as the biodegradation proceeds. It is highly unlikely, therefore, that it will ever be possible to bring about 100 percent biodegradation of crude oil. One recent approach to stimulating asphaltene degradation is to use oxidizing agents such as peroxide to introduce molec ılar oxygen into the asphaltenic molecule, thereby enhancing its biodegradability.

Water insolubility

Oil-degrading micro-organisms usually grow at the interface between the bulk aqueous phase and the oil layer floating at the surface. In fact, they are frequently found sticking to the oil layer. This direct contact between the organism and the oil growth-substrate represents one way in which these organisms have adapted to growth on the highly insoluble carbon source.

One of the problems the microbe faces when it occupies the surface of the oil is that it rapidly becomes depleted of a surface on which to grow. One way to enhance the growth of oil-degrading microbes is to increase the surface area of the oil. Enhancement of growth on oil can be accomplished artificially by the addition of chemical surfactants or detergents that cause the breakdown of the oil layer into small droplets, thereby increasing the surface area. This approach is effective provided that the added chemicals are not toxic to the organisms in question (13).

It should be noted that the oil-degrading bacteria themselves frequently produce their own surfactants (biosurfactants), which both increase the surface area for growth and aid in the uptake of the oil (13). Thus growth of micro-organisms on oil is almost always accompanied by the dispersion of the oil throughout the bulk aqueous phase. This results in the removal of oil from various surfaces – a kind of cleaning action.

As products of microbial fermentation, biosurfactants are of commercial interest for application in a number of industries. They are frequently highly efficient, non-toxic, biodegradable surfactants, and many have been produced on an industrial scale. Their production by oil-degrading microbes may require growth of the micro-organism on hydrocarbons, or it may be independent of the carbon source (14).

Physical state of the oil

Crude oil and petroleum products introduced to the marine environment are immediately subject to a variety of physical and chemical changes (see Figure 1). Among these changes are major non-biological weathering processes including evaporation, dispersion, formation of water-in-oil emulsions, adsorption of oil onto suspended particulate material, sinking, and sedimentation. In addition, there is ingestion by marine organisms and even some biodegradation. These processes occur simultaneously and cause important changes in the chemical composition and physical properties of the original oil, which in turn may affect the rate or extent of biodegradation of the pollutant (5).

Weathered oil (after evaporation of the light fractions) may contain about 10 percent low molecular weight alkanes, 45 percent paraffinics of higher molecular weight, 5 percent light aromatics, 20 percent of the higher molecular weight aromatics, and 20 percent asphaltenes and resins (6).

Heavy wave action and turbulence often give rise to the entrapment of large amounts of water within the insoluble oil layer. These water-in-oil emulsions, often termed "mousse" because of their physical appearance, may consist of as much as 30 to 50 percent water by weight; commonly, the heavier or more viscous the oil, the more stable the mousse. The oils present in mousses are particularly resistant to microbial attack, owing in part to the low flux of oxygen and nutrients to the aqueous phase.

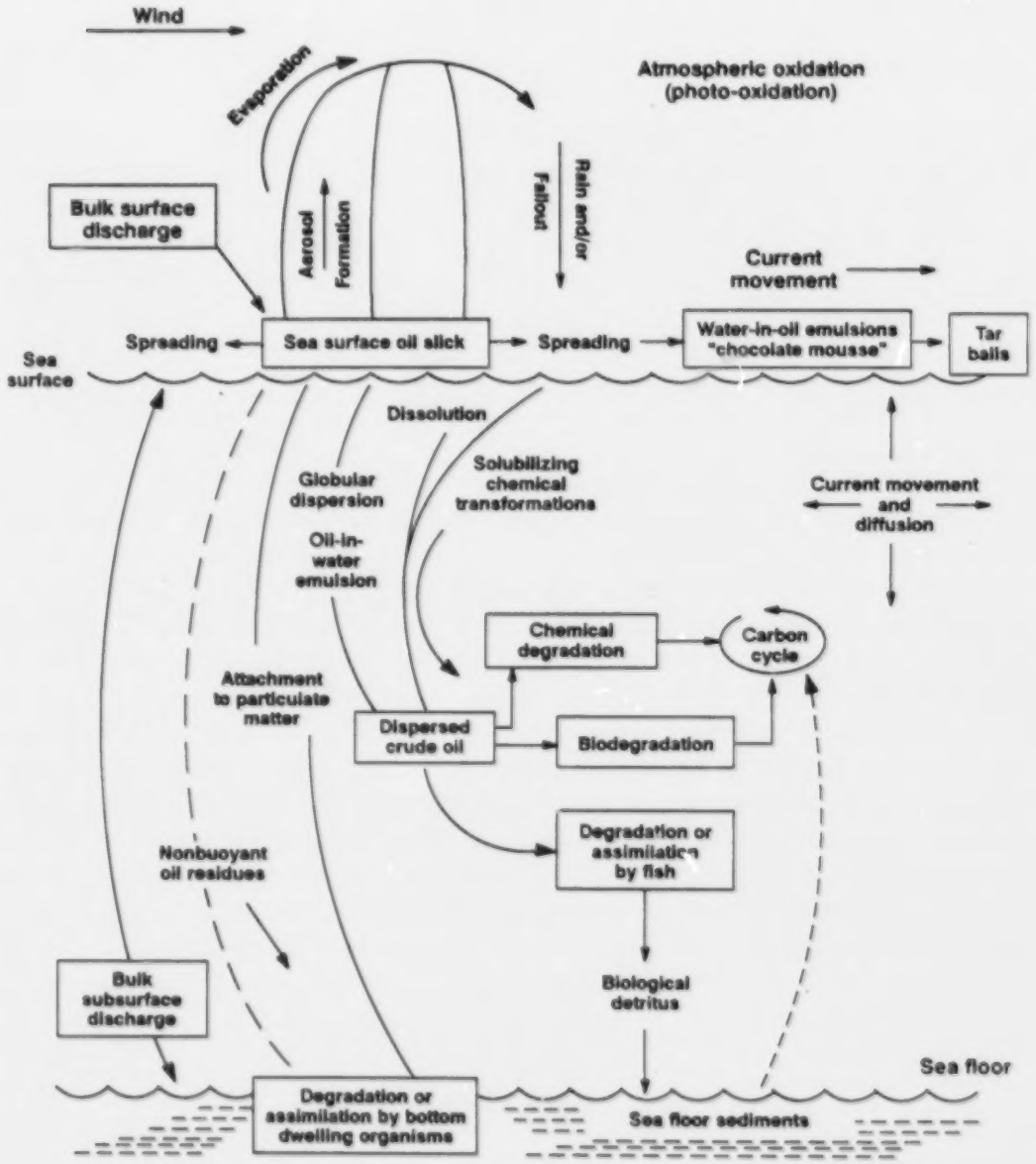

Figure 1 – Schematic of physical, chemical, and biological processes

SOURCE: National Research Council, *Oil in the Sea: Inputs, Fates, and Effects* (Washington, DC: National Academy Press, 1985), p. 271. Adapted from R. Burwood and G.C. Speers, "Photo-oxidation as a Factor in the Environmental Dispersal of Crude Oil", *Estuarine Coastal Marine Science*, vol. 2, 1974, pp. 117-135.

2.1.4 Other factors affecting biodegradation of oil

Temperature

Biodegradation of oil has been observed at most seawater temperatures from -2°C to 35°C. While the pattern of degradation has not been found to vary significantly, the rates of degradation are highly temperature-dependent, so that a drop in temperature from 25°C to 5°C brings about a tenfold decrease in rate. At lower temperatures, the rate of evaporation is significantly lower, so that lighter fractions may persist in the water for longer periods. Lower temperatures also increase the viscosity of the oil and lower its dispersability, so that the surface area for degradation is much reduced. This lowers the degradation rate as well as the extent of oil biodegradation (6,15).

Concentration of oil

Laboratory experiments have shown that rates and extent of oil degradation are limited by the concentration of the carbon source in the culture. For example, an oil-degrading bacterium isolated in the author's laboratory exhibited a generation time (the time required for one microbial cell to grow and divide into two cells) of 75 minutes when growing on 5 percent heavy Mexican crude oil. However, when the oil concentration was raised to 25 percent, the generation time was increased to 130 minutes[2]. Many other investigators have made similar observations. This sensitivity may be due to the presence of toxic materials in the oil growth substrate (16). This finding implies that bioremediation probably will not occur in "oil lakes" or heavily contaminated soils. It appears to work best once the bulk of the oil is removed by other means.

2.2 The technology

The application of bioremediation to the treatment of marine oil spills, it is important to note, represents but one application in a whole series of environmental problems. For example, bioremediation efforts are currently being considered or have already been applied to a number of other environmental problems including:

- treatment of toxic or hazardous wastes,
- treatment of contaminated ground water;
- decomposition of greases and viscous materials; and
- treatment of non-toxic solid and liquid wastes (5,17,18).

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[2] *Gutnick and Reisfeld, observations.*

In all these cases, the approach is the same: to generate the appropriate conditions and supplement the environment with suitable chemicals and sometimes micro-organisms to bring about the biodegradation of the oil pollutant.

In terrestrial environments much attention has been given to the process of land farming, in which the oil pollutant is simply ploughed into the upper layer of the soil in the presence of fertilizers, and bioremediation is allowed to occur. The process results in the accumulation of inert asphaltenes, which appear not to affect the subsequent growth of crops (19). This process has been banned by the EPA in the United States, but is still allowed in Canada. Disadvantages to land farming include the following:

- the uncertain fate of polyaromatic hydrocarbons (PAH) in the land farm environments;
- land farming of oily waste is likely to be banned throughout Canada within the next few years;
- the technology is applicable only to contaminated soil in which the pollution is located high enough to be ploughed in order to achieve sufficient exposure to oxygen;
- the remediation takes place in open systems in which seasonal temperatures limit both the rate and extent of oil degradation.

While much progress has been made in developing technologies that utilize bioremediation in the treatment of terrestrial environments in closed or semi-closed containers (bioreactors) where conditions can be continuously controlled and monitored, much less has been achieved in practically applying bioremediation in marine environments. Nevertheless, in 1989, following the *Exxon-Valdez* oil spill, a joint effort by the U.S. EPA, Exxon, and the Alaska Department of Environmental Conservation (ADEC) was mounted to conduct what remains the largest and most comprehensive series of experiments on oil-spill bioremediation. A summary of the bioremediation project in Alaska and the problems associated with the interpretation of many of the results was presented at the TDC Workshop on Bioremediation of Marine Oil Pollution in October 1991 by Professor Ron Atlas. This material is presented in section 6.2.1 of this report. Summaries of the Alaska bioremediation efforts have been presented in EPA reports as well as in the October 1991 issue of *Scientific American* (1).

In considering the biodegradation process, three basic approaches to bioremediation of oil pollution have been proposed:

- nutrient supplementation and enrichment of indigenous oil-degrading micro-organisms;
- addition of cultures of oil-degrading micro-organisms previously isolated from natural environments along with the nutrients – a process often referred to as seeding; and
- addition of pure cultures of genetically engineered micro-organisms "constructed" in the laboratory along with the nutrient supplements.

2.2.1 Nutrient limitation

It is commonly accepted that the major factor controlling petroleum biodegradation in marine environments is the availability of nitrogen, phosphate, and occasionally trace metals. Thus, any technology for bioremediation must involve supplementing the environment with these essential nutrients. In open aqueous systems classical sources of nitrogen (ammonia or urea-based fertilizers), as well as phosphate salts, tend to be diluted and washed away. However, in the 70s Atlas and Bartha introduced the concept of substituting the normal N and P nutrients with materials that tend to adhere to the oil. Such fertilizers have been termed oleophilic ("oil-seeking") fertilizers. Compounds such as paraffinized urea, octyl phosphate, ferric octoate, and paraffin supported $MgNH_4PO_4$ have been found to stimulate crude oil biodegradation in the sea and in Arctic ponds. The notion of supplementation with such materials was ignored for many years until the *Exxon-Valdez* spill, after which bioremediation was attempted along with hot water washing, chemical dispersion, physical containment and recovery, and stone polishing. Table 1 gives a partial list of shoreline clean-up techniques. The beaches in Prince William Sound were particularly suited to bioremediation studies since they were found to be relatively high in initial numbers of oil-degrading bacteria, quite low in nitrogen and phosphorous, highly porous, and subjected to large tidal fluxes that allow for strong mixing and aeration.

In the beach trials in Alaska, several types of fertilizers and application techniques were tried (5). These included slow-release, water-soluble fertilizers in both briquette and granular forms; a water-soluble fertilizer applied with a sprinkling system; and an oleophilic fertilizer, Inipol EAP22, a product of the Elf Aquitaine Corporation formulated with a solvent carrier to maintain the contact between the oil and the fertilizer. The EPA determined that the most successful and efficient way to treat the oily beaches was to use the Inipol spray on beach surfaces and to use slow-release fertilizers and oleophilic fertilizers for surface and subsurface oils. Many of the components of Inipol EAP22 are effective surfactants in addition to serving as oleophilic fertilizers. It has been contended that the clean appearance of the beaches following fertilizer supplementation may have been due more to the

Table 1 — Partial list of shoreline countermeasures

FLOODING COLD-WATER, LOW-PRESSURE WASHING HOT-WATER, HIGH-PRESSURE WASHING STEAM CLEANING WATER INJECTION SEDIMENT RELOCATION FOR NATURAL CLEANING TILLING EXCAVATION AND REMOVAL ROCK-WASHING RAKING MANUAL PICK-UP WIPING CLEANING AGENTS WETTING AGENTS SINKING AGENTS DISPERSANTS SOLIDIFIERS IN SITU BURNING INCINERATION SORBENTS CLAY "SCAVENGING" GEOTEXTILES
BIOREMEDIATION
COMBINATIONS OF THE ABOVE

detergent effects of the Inipol than to the biodegradation it was thought to stimulate. The apparent effectiveness of water-soluble fertilizers in the trials suggests, but does not prove conclusively, that the fertilizers did in fact stimulate the bioremediation. It must, however, be emphasized that each polluted environment is likely to respond differently, and the details of the nutrient delivery technology (form and type of fertilizer, type of application, number of applications, form of monitoring, evaluation of outcome, etc.) may need to be established for each site.

2.2.2 Open vs closed systems

Once oil is spilled onto the sea, the slick can spread extremely rapidly, depending on the weather conditions, wave action, etc. Physical containment of oil under these conditions is minimal. For example, only about 10 percent of the spilt oil was physically recovered after the *Exxon-Valdez* spill[3]. The remainder underwent severe weathering and eventually settled on the beaches where it was treated as described above. Were the technology available, successful bioremediation at sea could have led to significant enhancement of the oil breakdown, and perhaps even shortened the period of remediation efforts once the oil reached the shore.

2.2.3 Seeding

Naturally occurring oil-degrading micro-organisms

Seeding involves the introduction of specific microbial populations into the environment for the purpose of increasing the rate and/or extent of the biodegradation of pollutants. The rationale for this approach stems from the fact that although a wide range of potential substrates present in oil may not be degraded by the indigenous populations, they can be degraded by selected pure or mixed cultures. Note, however, that with seeding the environment must still be supplemented with N and P. The complex composition of oil requires that seed cultures consist of consortia. These consortia should be non-pathogenic and stable under long-term storage conditions, and they may need to be able to compete successfully with indigenous micro-organisms. In addition, the chosen consortium should be physically compatible with the hydrophobic (and frequently chemically dispersed) oil, so that it can be applied evenly to the oil slick. Although it is commonly accepted that bioremediation is fruitless in the open sea, one advantageous technology for bioremediation in open systems could involve production and delivery of suitable "tailor-made" oil-degrading consortia that meet the above criteria and are formulated to carry the necessary nitrogen and phosphorous nutrients. For those environments poor in indigenous oil-degrading populations (such as the open sea), micro-encapsulation technology could be adapted to produce oleophilic microbial capsules that carry nutrients and begin to release their contents upon contacting the slick.

Genetically engineered micro-organisms

The application of recombinant DNA technology to microbiology has been at the forefront of biotechnology for the past 15 years. In fact, the very first micro-organism to be patented

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[3] *Captain William Ryan, personal communication.*

was a genetically engineered strain of Pseudomonas "engineered" to degrade oil (20-23). This engineered organism has not been shown to degrade oil any better than naturally occurring populations, nor has it been subjected to any field trials. Nevertheless, proponents of this technology continue to argue that genetic engineering and recombinant DNA may lead to the creation of organisms that can degrade the more recalcitrant molecules, or to strains that can degrade other fractions of oil more efficiently than naturally occurring strains. It is now commonly accepted that the degradation of complex heterogeneous petroleum substrates requires the action of consortia rather than a single specific strain. The development of genetically engineered organisms for application to oil spills would appear to be unnecessary (5). The application may be useful in the future for dealing with specific chemical spills in which a single pollutant may need to be disposed of, but the engineered organism will still have to overcome the environmental and nutritional limitations required of the naturally occurring bacteria.

2.3 Advantages and disadvantages of bioremediation

The advantages and disadvantages of bioremediation technology for marine oil spills, outlined in a recent report on behalf of the U.S. Congress, Office of Technology Assessment, are summarized in Table 2.

The first advantage of bioremediation, and perhaps its most important feature, is the relatively small effect it has on the environment. When compared with either high-pressure washing, in particular the use of hot water washes, or mechanical clean-up of what were once pristine beach sites, bioremediation technology as applied in Alaska had little or no impact on the physical integrity of the clean-up site. Second, experiments in Alaska suggested that there were no damaging or long-lasting toxic side effects. A third advantage may be that rapid degradation prevents the accumulation of the more toxic elements in petroleum, such as the aromatic or polycyclic aromatic hydrocarbons. The capital outlay for bioremediation is certainly less than that for technologies involving mechanical recovery equipment. Since bioremediation of a beach site may involve little or no treatment other than spraying with nutrients, it could be less costly and less labour intensive than the washing technologies, which may involve flushing the oil back into the water, thus requiring subsequent recovery and separation technology. Of course, if bioremediation is used as a secondary or ancillary clean-up technology, the cost of the total clean-up effort could be increased significantly. Moreover, the chemical analysis and monitoring of the bioremediation effort entail additional cost.

The primary disadvantage of bioremediation in open systems is that it is time consuming, requiring days and even weeks to achieve maximum biodegradation. Thus it is not a

Table 2 — Bioremediation in open systems

ADVANTAGES	DISADVANTAGES
• MINIMAL PHYSICAL DISRUPTION OF SITE • PROBABLY LESS TOXIC • POTENTIAL DEGRADATION OF MORE TOXIC ELEMENTS IN PETROLEUM • MAY BE LESS COSTLY • MAY BE MORE THOROUGH THAN MECHANICAL RECOVERY TECHNOLOGY	• SLOW PROCESS • MUST TAILOR TECHNIQUE FOR EACH SITE • MAY NEVER GET 100% DEGRADATION • MAY NOT BE USEFUL IN OPEN AREA • PUBLIC ACCEPTANCE REQUIRES MANY FIELD TESTS OF: – Efficacy – Safety – Expense

short-term primary technology, but will likely be used in conjunction with other treatments designed to remove most of the pollution quickly. A second major disadvantage is that specific sites may require new adaptation. The rules linking the type of oil pollutant, the particular environment where the remediation is to take place, and the predicted rates and extent of bioremediation are still unknown. Thus each event must await a period of preliminary testing. Third, 100 percent biodegradation is probably not realistically achievable given our current understanding of microbiology. A fourth problem is that the technology may not be applicable to the open sea. Thus far, there is no field experiment demonstrating that bioremediation works in open aqueous systems. Finally, before the technology is accepted, field experiments must be performed to demonstrate both efficacy and safety to the public.

3. APPLICATION OF BIOREMEDIATION TO MARINE OIL SPILLS

In the 22 years between 1967 and 1989 there were 66 large oil spills (in excess of 2 million gallons) throughout the world (24). Of these, 44 were due to tanker collisions, groundings, breakups, fires, or explosions. The tanker spills off the coast of British Columbia, the *Mega-Borg* spill in the Gulf of Mexico, and the catastrophic intentional dumping in the Persian Gulf should be added to this list.

3.1 Oil spill scenario

Of the oil transported in coastal waters, crude oil is likely to be the most common in terms of volume, while bunker oil is transported more frequently (25). For example, risk analysis for oil spills along the Pacific Coast of Canada (26) shows that bunker spills are likely to be more frequent but smaller (in the order of 1000s of bbl), while crude oil spills are likely to be less frequent, but one to two orders of magnitude larger (10-100 thousand bbl).

Many spills result from either a grounding or a vessel collision near the coast, where only a small or moderate degree of weathering might occur before the oil reaches the shore. Therefore, heavy shoreline oiling might be expected within hours to days. This oil might be heavily emulsified before reaching the beach.

In high risk navigation areas, a crude oil spill is more likely to occur from a tanker during either a grounding or a vessel collision, while bunker spills might originate from a cargo vessel or barge spill.

In terms of environmental risks, the crude oil spill is considered the more significant for two reasons: crude oil is more difficult to clean because low viscosity allows for rapid spreading and penetration into porous beaches, generating subsurface oil pockets; and crude oil is more toxic than bunker oil.

In terms of biodegradation of the spill pollutant, however, crude oils, particularly the lighter crude oils, may be more easily biodegradable than the heavier bunker fuels.

As a spill response technology, bioremediation could, in principle, be applied either to an oil spill in the open sea or to the coastal region once the oil has reached land. Most experts expect that applying the technology to the open sea would not prove particularly useful or applicable. The basic problem appears to be testing the efficacy of such a technology in an open system when so many interferences can affect the results and their interpretation. How can one tell if it works? The open sea is simply an uncontrolled environment. It is

important to note that the only evidence in favour of bioremediation as an effective technology stems from experiments on shore, such as those carried out during the *Exxon-Valdez* incident, and the results from those experiments are not conclusive.

There are, however, obvious advantages to significantly enhancing oil biodegradation before the spill reaches the coast line. The major advantage is that, if successful, starting the bioremediation process at sea could enhance the overall rate of remediation once the oil reaches the shore. The following section, while speculative at best, considers the factors affecting the technology for such an application.

3.2 Bioremediation of marine oil spills at sea

To date no one has presented convincing evidence that bioremediation can be used in the open sea. The *Mega-Borg* spill in the Gulf of Mexico was treated with a mixed culture of oil-degrading micro-organisms and the spill was reported to have been significantly reduced in size within 16 hours. Unfortunately, no controls were provided to account for the mechanical disruption effects of waves and stormy weather in the Gulf of Mexico during the treatment. The claims to a successful bioremediation effort in the Gulf of Mexico have not been taken seriously by responsible professional groups such as the American Society of Microbiology (5).

Another attempt at bioremediation was made when the Greek tanker *Shinoussa* collided with three barges in the Houston Ship Channel, resulting in a spill of about 700 000 gallons of a partially refined catalytic feed stock. Part of the spill settled in a salt marsh and microbes were applied to experimental areas within the marsh. Other areas were left untreated. Within a few minutes to a few hours after treatment, the oil in the experimental plot was found by the government observer to have changed colour, which was taken as some indication of microbial action on the oil. However, within a few days there was no significant difference in colour of the spilt oil between the experimental and the control areas. While the data are still undergoing evaluation, there is no evidence to indicate that the seeding had any effect.

3.2.1 Remediation scenario

The best case situation for the use of bioremediation in a marine spill at sea would most likely involve the use of liquid sprayable formulations containing either nutrients alone or nutrients along with bacteria to be sprayed from the tanker itself or from suitable aircraft. If the spill is due to a relatively slow leak, the material stored as liquid on board could be

added directly to the leaking tank(s). In that way both the oil and the nutrients would reach the sea together. This would not be appropriate if the leak is reparable, or the tank sealable. The enhanced degradation could begin immediately and the overall process continue even after the oil washes ashore. Additional spraying cycles could then be carried out at regular intervals by aircraft. Even if the bioremediation was not complete by the time it reached the shore, it could have an enhancing effect on the subsequent remediation on the beach.

Although the above picture seems relatively straightforward to develop as a technology, there are numerous important technical constraints and barriers to overcome. These are discussed briefly in Section 3.2.2.

3.2.2 Technical barriers

Delivery of nutrients to the site

Fertilizers (oleophilic) must be delivered to the spill site as soon as possible after the oil reaches the water. Since spray systems are already available for applying chemical surfactants and dispersants to an oil slick, they might be used for nutrient delivery as well. When the spread of the oil or its leakage from the vessel is relatively slow, the nutrients might be sprayed from the ship itself. A more usual situation, particularly in the case of a stormy sea or rapid spreading, would probably involve spraying from aircraft or helicopters, which would have to obtain materials from stockpiles in warehouses. The problem in this situation is the targeting of the spill. The problems are akin to those found with the targeting and delivery of chemical dispersants to oil spills. For an *Exxon-Valdez* type spill, oil can spread to an area of about 6 square miles within the first 12 hours, and by 4 days can spread up to about 17 square miles (this assuming no wind or current). It should be noted that with time the slick tends to fragment and break up into patches, which can increase the total surface area significantly.

Choosing appropriate oleophilic fertilizers

Oleophilic fertilizers will probably be used in the open sea since they are more likely to adhere to the oil and, therefore, less likely to be washed away. The best studied of the oleophilic materials is Inipol. While shown to be somewhat effective in Alaska, Inipol did not enhance biodegradation of oil when added to an oil spill at sea. Nor was it effective in other sites (5).

One possibility is that Inipol, which contains oleic acid as a mild surfactant, may be degraded as a carbon source in place of the oil, and the nitrogen metabolized by the irrelevant organisms. This was found by Lee and Levy (27), who showed that Inipol was less

effective in bioremediation on beaches than were agricultural fertilizers. They observed a significant loss in Inipol nitrogen content. The nitrogen (fertilizer) to carbon (oil) ratio has a significant effect on the growth rate of the degrading organisms. With Inipol, this ratio can be predicted and controlled in closed environments, but may not be maintained in open systems. Clearly, a search should be conducted for other, perhaps less expensive, oleophilic fertilizer formulations that adhere strongly to the oil but do not present the same difficulties as Inipol.

Adaptation and stabilization of consortia used in seeding

Oil-degrading microbial populations are present in almost every environment where they have been sought. Therefore, they will probably be present in shipping lanes where accidental spills, intentional dumping, natural off-shore seepage, and collisions occur. Nevertheless, when oil is spilled into the sea, there is likely to be some adaptation period before the active bioremediating populations take over (6). Proponents of seeding would hope to eliminate this period by supplementing the environment with suitably adapted organisms along with the nutrients. This time factor might be of great significance in affecting subsequent shore clean-up (if necessary).

Ideally, the populations of the seed cultures should be prepared fresh from growing cultures immediately prior to use. In fact, the group from Alpha-Microbiologicals that hoped to use their cultures in the Persian Gulf expected to be able to grow up to 30 barrels of microbial culture aboard a barge. No data are available as to the time it would take to produce the cultures in large enough quantity, but according to the author's experience this is likely to be a question of days rather than hours. In an emergency situation, however, it is unlikely that a fermentation of this magnitude would be possible on board a tanker with troubles of its own; and also unlikely that a vessel equipped with bioreactors could get to the site of the spill in time to provide the large volume of microbial culture needed to eliminate the period of adaptation. What is likely to occur in these situations is that the microbes, if they are to be added, will be added in the form of freeze-dried powders, and may themselves require a period of rejuvenation and adaptation before becoming effective. In addition, cultures prepared by freeze drying (a process termed lyophilization) frequently lose viability, so that the actual percentage of living organisms in the powder may be only 0.1 to 1 percent of the initial input. In this case the time-factor advantage may be severely reduced.

To deal with this problem it may be necessary to find ways of storing the microbial consortia in a powder or semi-solid state so that they can be stored and can retain viability for long

periods, and can be added along with the requisite nutrients. One technology that might be adopted from other applications is the use of microencapsulation to prepare small microbial "packages" containing nutrients and preadapted consortia that would adhere avidly to the slick when applied and remain stuck to the oil as the biodegradation occurs. The encapsulation of the organisms along with the nutrients would allow better contact between the fertilizers and the hydrophobic substrate.

Many currently operating companies are producing microbial consortia to enhance bioremediation of oil spills. The preparations are often applied together with their own nutrients. However, it should be stated categorically that not one single product of this kind has been shown to work for open systems. Frequently the construction of these consortia involves combining microbial strains from around the world, each of which is responsible for a different metabolic activity. Therefore, the use of these seed cultures involves the introduction of foreign micro-organisms into the environment. In many cases, the reconstructed consortia have never been tested under the conditions of a marine oil-spill environment. Currently the EPA and Environment Canada are developing suitable test protocols for the evaluation of these biological products. It is likely that these cultures will also need to be accepted by a series of regulatory agencies before they can be released into the environment.

3.2.3 Evaluating performance

A major difficulty in testing bioremediation packages in open sea environments lies in the uncertainty of the results. How are the results to be monitored? (Consider the *Mega-Borg* incident, for example.) How does one design a field experiment? Probably the reliability of interpretation will be limited by a number of interfering factors, such as the spreading of oil, rapid weathering, fragmentation of the slick, and formation of "chocolate mousse", as well as by the physical effects on the oil resulting from the presence, in the bioremediation formulation, of surfactants associated with the fertilizers. Moreover, rare cases have been documented in which favourable winds and currents caused the oil to be carried out to sea rather than to approach the shore (21).

Chemical Analysis

One common approach to identifying bioremediation is to analyse residual oil from a bioremediation experiment and compare it with similar oil from an untreated control. If biodegradation is occurring in one of the samples, the degraded fractions should decrease considerably or even disappear when compared to a molecule present in oil, which is normally considered to be recalcitrant to biodegradation (an internal standard).

One problem discovered in the *Exxon-Valdez* clean-up was that molecules such as n-C_{17}: pristane or n-C_{18}: phytane, which were thought to be only slightly degraded by bacteria, were actually degraded in Alaska at a rate similar to other molecules (5). Thus, the ratio obtained was not amenable to interpretation. A search is under way among analytical chemists and microbiologists to identify appropriate internal standards to gauge the extent of oil degradation.

Extent of degradation on shore after degradation at sea

Any spilt oil reaching the shore is likely to be partially weathered by physical effects and perhaps by natural biodegradation as well (although this effect is likely to be relatively small). It would be of interest to determine if prior bioremediation or enhanced biodegradation of oil at sea could lead to an increased rate of breakdown after the oil reaches the shore. Future field experiments for bioremediation experiments on beaches might contain additional plots to study different forms of oil (emulsified, partially biodegraded, etc.) as substrates for bioremediation.

3.3 Bioremediation of marine oil spills on shore

Shores and coastlines are considered to be more suitable sites for bioremediation of oil spills than the open sea (5). In part the reason stems from the ease of targeting the sites for clean-up. In addition, if the spill is near the shoreline and the drift is toward shore, it will be almost impossible to prevent beach contamination, no matter how ideal the conditions.

Another reason to consider bioremediation on shore is that the contact of sediments with the oil may frequently bring about increases of several orders of magnitude in the number of hydrocarbon-degrading micro-organisms. This was found for the *Amoco-Cadiz* spill off the Coast of Brittany (28, 29). In addition, in some cases, new and interesting organisms can be identified. It was found, for example, that populations of micro-organisms in Antarctic sediments had greater degradation potential for *Metula* crude oil at 3°C than at 22°C (6).

Although practical applications of bioremediation of major spills on land or at sea have been attempted in a few instances (see above), most of the results of bioremediation stem from large-scale field experiments on land. Moreover, the largest field experiment in bioremediation to date, that conducted during the *Exxon-Valdez* clean-up, was not the result of an intentional experimental spill. As such, many uncontrolled variables were inadvertently introduced into the system during the experiment, making a precise conclusion difficult.

3.3.1 Pitfalls in field trials

The *Exxon-Valdez* field experiment in bioremediation of oil pollution was discussed in detail by Dr. Ronald Atlas as part of his keynote presentation at the TDC Workshop on Bioremediation of Marine Oil Pollution held in Montreal in October 1991. In addition, the discussion group dealing with field experiments listed and discussed many of the interferences to good measurements in field experiments (see Table 10). Some of the points outlined below are abstracted from this material.

The results of the *Exxon-Valdez* experiment showed that beaches treated with fertilizers were significantly cleaner than similar plots that were not so treated. Moreover, some data supported the theory that there was enhanced microbiological activity in the fertilized plots. For example, total bacterial numbers increased, microbial metabolic activity measured as oxygen uptake was elevated, numbers of hydrocarbon-degrading microorganisms increased, and chemical analysis indicated a significant disappearance of hydrocarbons (5). Moreover, the oil that was degraded was actually removed from the site and never returned. Why are the results considered inconclusive?

Oil disappearance

The fertilizer Inipol contains a mild surfactant. Some have suggested that this material could in fact carry out some chemical rock washing. However, anecdotal field evidence together with laboratory experiments preclude this possibility[4]. Others have suggested that rather than support the growth of bacteria, Inipol, as a fertilizer, promotes the removal of oil from rock surfaces by enhancing a process of co-settling (termed flocculation) that occurs between oil and fine clay particles. An alternative hypothesis of the author, which has not been disproved, is based on the observation that Inipol itself is degraded by other organisms (30). Some of these may produce biosurfactants or flocculants that interact with the oil as described above.

In addition, it is known that, by causing the breakdown of the oil into small droplets, a surfactant can increase the surface area of the oil, which may in turn result in increased rates of evaporation and weathering. Thus, the disappearance of the oil in response to fertilizer need not imply enhanced bioremediation if the fertilizer contains surfactant.

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[4] *Dr. R. Atlas, personal communication.*

Analytical problems

The measurement of oil disappearance by physical loss depends on extracting the oil from a sample of sediment, etc., into an organic solvent and measuring the absorption of the organic extract by IR or by UV fluorescence spectroscopy (31). If the oil is complexed somehow in a strong emulsion generated by surfactants or detergents, more rigorous extraction procedures must be used. Otherwise, the apparent residual oil in the sediment may be estimated at too low a level. In Alaska, hexane was used as a solvent rather than the more efficient dichloromethane. Hexane is not sufficiently effective in extracting all of the oil from a strongly emulsified complex.

Sampling is also a problem on a cobbled rocky beach where tens of thousands of rocks of different sizes are polluted with varying degrees of oil. In monitoring residual oil, it was necessary to choose a realistic number of rocks for extraction and to determine an "average residual oil per rock". The techniques are arbitrary at best.

Unintentional "stacking of the deck"

During the course of the experiment in Alaska, an intensive effort of manual rock and stone cleaning was under way. In some cases, workers inadvertently "invaded" experimental plots and cleaned the area by hand. Moreover, certain areas that should have been chosen as lower energy oiled plots could not be used. These regions could not be staffed sufficiently because of the danger to the workers of invading bears. Finally, it should be noted that, fortunately, the spiller was able to bear the cost of the $2.5 billion clean-up effort for the *Exxon-Valdez* spill. There is no question that the company is interested in presenting the results of the bioremediation experiment in Alaska in the best possible light.

3.3.2 Field trials

It is commonly accepted amongst a number of experts that the best way to evaluate oil spill clean-up technology is to carry out a relatively large-scale simulation under conditions approaching those that may be present during a real spill. An overview of one such major field experiment is presented in references 32 and 33. Moreover, experience has proven that results from realistic relatively large-scale field tests will be required for public acceptance of bioremediation. Intentional spills are not permitted in the United States and other countries, but they have been allowed in Canada. The larger-scale trials have included the cooperation and partial funding of other countries such as Norway, the United

Kingdom, and the U.S. In the case of the Baffin Island Oil Spill (BIOS) project, about 25 percent of the funding was foreign, with the remaining 75 percent divided equally between the Canadian government and the oil industry.

In the following sections, some examples of experimental spills, their design, and their results regarding bioremediation of oil will be briefly considered. Space does not permit a comprehensive treatment of these examples. They are presented here primarily because they represent design models likely to be used in future larger-scale field trials (22). The reader is referred to the reports and publications presenting the detailed results.

The BIOS project (32, 33)

The BIOS project was a multidisciplinary study of an experimental spill whose major objectives were to assess the chemical and physical fate of oil in Arctic coastal areas; to assess the effects of chemical dispersants on the biological effects of oil; and to examine the effectiveness of various shoreline clean-up techniques. Begun in 1980 and proceeding through 1983, the project was an international cooperative effort managed by Mr. Gary Sergy of Environment Canada. Limited annual surveys were continued for an additional five years. The experiment included a near-shore study and a shoreline study.

In the near-shore study two oil slicks of 75 barrels each were released into similar bays, while a third untreated bay served as a control. One of the slicks contained untreated oil, while the other was treated with chemical dispersant.

The shoreline cleaning study involved oiling control plots in 1980 and during the second and third years oiling additional plots in which different beach-cleaning methods were tried. The crude oil was the same as that used in the near shore study. In some cases oil was also added as "chocolate mousse". After oiling, the plots were left for 24 hours before initiation of clean-up. It was found that in areas of high wave energy over 99 percent of the oil was removed within 48 hours. Sheltered beaches lost most of their oil within a season, and by the second year more than 99.5 percent had disappeared. Backshore beaches without exposure to wave action showed no changes except for the formation of a layer of tar. Chemical dispersants seemed to accelerate the rate of oil disappearance from rocks and cobbled areas. Burning was found to be ineffective because of the difficulty of keeping the oil ignited.

The microbiological studies (34, 35) included examination of the effect of oil on the activity of the total heterotrophic population and the effect of addition of fertilizers on the rate of oil degradation. Oil affected the behaviour of the total heterotrophic population very little.

Following the addition of a commercial fertilizer, however, an increase in the number of alkane-degrading bacteria was observed and degradation of 30-60 percent of the biodegradable alkane fraction occurred in one year. Mechanical mixing of the oil and fertilizer into the sediment seemed to enhance the rate of biodegradation.

Bioremediation of Scotian Shelf condensates and waxy crude oils (Terra Nova) on low-energy shorelines in Nova Scotia (27)

The need for accurate determination of the correlation between microbial activity and oil biodegradation *in situ* prompted Lee and Levy (16) to undertake a series of studies to specifically assess many of the rate limitations to bioremediation in the field.

In one set of experiments a light Scotian Shelf condensate (SSC) was tested for biodegradability at a site in Long Cove on the eastern shore of Nova Scotia for 204 days. This SSC was found to be toxic to micro-organisms, and bacterial growth in the oiled sediment did not occur until evaporation or weathering of the light aromatic fractions occurred. At low temperatures the time delay for this weathering could be quite significant. The addition of Inipol had very little effect on the biodegradation, although it did seem to enhance degradation in the laboratory. Repeated doses of less expensive agricultural fertilizers were more effective in enhancing biodegradation.

In another set of field experiments at Long Cove and Clam Harbour in eastern Nova Scotia, Lee and Levy monitored the degradation of the waxy Terra Nova crude oil both in sand marshes and on a rocky beach. In an oil concentration of 0.3 percent (3000 ppm) degradation took place on the sandy beach in a matter of days. Nutrient addition had very little effect under these conditions. However, in the presence of a tenfold higher concentration of oil, almost no degradation occurred in the absence of added nutrients. When fertilizers were added, biodegradation was significantly enhanced, such that residual oil was in the order of 2-17 percent. In the salt marsh experiment, almost no biodegradation occurred in the absence of added nutrients, even at low oil concentration. The addition of fertilizers to sensitive ecological sites such as salt marshes may serve as an effective bioremediation treatment of oil pollution. However, the time scale in these experiments is weeks and months rather than hours and days.

More recently, Dr. Lee has proposed using materials such as heat-treated peat (in which the hydrophobic waxes are driven to the surface) in conjunction with agricultural fertilizers to increase the surface of the oil-microbe-nutrient interface, thereby increasing the rate of bioremediation. Preliminary laboratory studies have demonstrated significant

increases in the natural biodegradation rates of Beaufort Sea crude oil impregnated into beach sediments from Long Beach, Vancouver Island, following treatment with nutrient-supplemented oleophilic peat.

Preliminary experimental design for a Pacific Coast oil spill

The shipping lanes of the Pacific Coast are recognized as high risk areas for major oil spills (26). As part of an effort to improve marine spill response capabilities in Canada, an experimental Pacific Coast Oil Spill is being planned for 1992 (22). The overall objective of this experiment is to "assess environmental impacts and effectiveness of promising clean-up options for coarse or mixed-sediment beaches" (22).

The concept of the experiment is the same as that applied in the BIOS project. The results of the BIOS project are frequently used for response planning for Arctic waters, since they allow a realistic evaluation of countermeasures in terms of both clean-up effectiveness and environmental impact. The Pacific Coast spill is designed to provide a similar capability for the West Coast.

Although the site has yet to be determined, the design calls for a series of intentional small-scale spills onto the intertidal zone of beaches or small bay shorelines. One oiled beach will be left untreated. In addition, a series of sites will be exposed to the clean-up methods without oiling in order to assess the impact of the countermeasures themselves. During the execution and monitoring of the experiment a "decision-tree" will be used to assess the efficacy of each countermeasure when compared with the environmental impact trade-off. The major issues to be addressed in the experiment include:

- countermeasure environmental impact;
- countermeasure efficacy;
- subsurface oil;
- bioremediation.

The countermeasures to be investigated include:

- flooding and washing – high pressure hot water and low pressure cold water;
- tilling – treatment of subsurface oil to bring it to the surface where it can be removed;

- beach-cleaning agents – used either in a prewash soak or as additives to the water during washing;
- bioremediation – effects of fertilizers, seeding, etc.

The proposed experiment calls for carrying out both crude and bunker oil spills with an option to use emulsified oil as well. The author has not seen the complete design of the experiment. However, a list of experimental priorities has been determined. If only one kind of oil is to be used it is to be crude rather than bunker oil. In terms of the countermeasures to be examined, flooding-washing and tilling are given higher priority than either bioremediation or the beach-cleaning agents. The author has learned that the bioremediation experiments are likely to be designed and conducted by the U.S. EPA.

4. ALLIED TECHNOLOGIES THAT AFFECT VARIOUS ASPECTS OF TRANSPORTATION

Bioremediation of oil spills is only one of many biotechnologies that can affect a host of environmental issues in the transportation industry. In this section the status of a number of such allied technologies will be considered, including bioremediation in closed systems and bioreactors, upgrading of fuels, biologically induced corrosion, and bioremediation in airport environments. The rationale for choosing these areas stems in part from the impressions of the various R&D activities observed by the author during his visits throughout Canada.

4.1 Bioreactors

Bioreactors are chambers that permit the cultivation of micro-organisms or the carrying out of biological reactions under controlled environmental and nutritional conditions (17). Much of the success of modern biotechnology and industrial microbiology depends on the design and control of such bioreactors. In clean-up of environmental pollution the use of bioreactors for biodegradation and bioremediation offers a number of advantages not normally associated with the simple addition of nutrients with/or without micro-organisms to open systems. A number of the advantages and disadvantages of using bioreactors in bioremediation treatments are listed in Table 3.

4.1.1 Systems that can be retrofitted

Common sites frequently contaminated with residual oily sludges and sediments are oil storage containers in ships and on land. To make ordinary repairs in both these systems, routine pollution clean-up is often required. The containers must be made gas-free so that welding torches can be used without risk of explosion. Moreover, moving or even scrapping storage tanks can be a major problem unless the sludge is removed and the tank bottoms are cleaned. Once the bulk of the oily waste is removed, the containers might be thought of as bioreactors for the remediation of the residual sludge and bottom deposits. The feasibility of this approach has been demonstrated in field experiments both on a ship and on land (36, 37, 38). To convert existing tanks to bioreactors requires the installation of some sort of piping system, so that air can be introduced to the tank; and of some type of heat exchange device, so that the water temperature can be maintained at 30–35°C. The bubbling of air into the tank can usually provide sufficient mixing.

Table 3 – Bioreactors in bioremediation

ADVANTAGES	DISADVANTAGES
• OPTIMAL CONDITIONS FOR OPERATION – Nutrient addition and pH control – Temperature control – Mixing • EITHER STATIONARY OR MOBILE UNITS • CONTROLLED OPERATION • EASE OF SAMPLING AND MONITORING • POSSIBLE APPLICATION IN BATCHWISE OR IN CONTINUOUS MODES • POSSIBLE ADAPTATION THROUGH RETRO-FITTING OF EXISTING SYSTEMS – Ship ballast tanks – Oil and fuel storage containers on land	• POSSIBLY NOT COST-EFFECTIVE FOR POLLUTION CONTROL • INITIAL INVESTMENT QUITE COSTLY • POSSIBLE PROBLEMS IN SCALE-UP • BIOREACTORS MAY BE DIFFICULT WITH TWO-PHASE SYSTEMS • LARGE VOLUMES OF PROCESS WATER FREQUENTLY REQUIRED

Bioremediation in an oil tanker

In this experiment a slop tank of about 200 m^3 volume containing approximately 1 percent oily sludge was cleaned during a ballast voyage of an oil tanker from Eilat to the Persian Gulf. The experiment was conducted at ambient temperature (26-32°C). The tank was filled with sea water supplemented with ammonium sulfate and potassium phosphate, and an inoculum of oil-degrading bacteria was added. Aeration was maintained at a pressure of 3-5 atmospheres. A control tank received no air, nutrients, or bacteria. The tank was cleaned within 72 hours, and could be dumped without the formation of an oil slick (Gutnick, D.L. and Rosenberg, E., U.S. Patent 3, 941, 692 – Cleaning of Cargo Compartments, 1976). Subsequent work showed that the culture formed in the slop tank could be used as an inoculum for the bioremediation of a regular-sized storage container. However, the remediation in this case was primarily oil emulsification and tank cleaning and de-sludging rather than complete biodegradation. Subsequent trials using microbial consortia in experimental tanks demonstrated a more complete biodegradation; residual oil (after weathering and evaporation) was degraded by about 85-90 percent.

The biodegradation of hydrocarbons is frequently accompanied by the production of acids that may in turn accelerate the corrosion process within the tank. This may be exacerbated by use of ammonium sulfate as a nitrogen source. In the author's laboratory it has been found that less severe fluctuations in pH within a tank undergoing bioremediation are obtained when urea is substituted for the ammonium salt. Although urea is somewhat more expensive than ammonium sulfate, the cost per nitrogen atom (urea contains twice as much N as ammonium sulfate) is about the same, and the effects less corrosive.

Bioremediation in oil storage containers

A second field experiment was conducted by the author in a Bunker C fuel oil storage container in an industrial site in Northern Israel. In this case the tank was about 20 m in length and only 85 cm high. This tank was filled to about 15-20 percent with sludge that had accumulated in the tank bottom over a period of years. The odd shape of the tank and its height precluded manual cleaning. The objective of the experiment was to clean the tank, rendering it gas-free so that it could be destroyed with a cutting torch and replaced with a more modern storage tank. Samples were removed from the tank and used in sequential enrichment cultures to isolate a microbial consortium that ultimately brought about the degradation of approximately 75 percent of the sludge. In this system the experiment was carried out initially at ambient temperatures (10°C at night and 22°C during the day). Because of the large fluctuations in weather conditions it became necessary to control the temperature. This was achieved by the introduction of steam into the tank and the manual monitoring of water temperature. The tank was cleaned and declared gas-free within a week. It was then dismantled with a welding torch.

In both the tanker and the storage tank the retrofitting was relatively minimal, requiring the installation of an aeration system capable of both aerating and mixing, and perhaps the introduction of a temperature-controlled system. However, in some instances, bioremediation may work best in a suitably designed dedicated bioreactor. The next section describes two such systems.

4.1.2 New types of environmental bioreactors

Mobile bioreactors[5]

The mobile bioreactor is a relatively new development in various European countries, particularly Germany. The mobile bioreactor is sufficiently large to process relatively

[5] *The author was introduced to this type of bioreactor during a visit to Germany in March 1991.*

large quantities of contaminated soil (17 m^3 per batch) while still being mounted on a self-propelled wheel-driven platform. It is designed to provide optimal temperature control, mixing, nutrient addition, and aeration, along with pH control. A total cycle time for a batch of oil-contaminated soil may be in the order of only a few hours. The concept of the mobile bioreactor is to bring the bioremediation equipment directly to the site(s) of pollution, rather than to haul large quantities of contaminated soils to a special site for either landfill or remediation. One problem recently discovered by German investigators working with a mobile bioreactor was that the growth of the micro-organisms was so extensive that they presented a good food source for various protozoa present in the soil. Within a couple of cycles the microbe population was almost completely devoured by these protozoa. Thus, for the bioreactor to be effective, it may be necessary to control the protozoan population. The second problem with the mobile bioreactor is the enormous cost of the equipment. The German bioreactor seen by the author costs about 6 million German marks.

The peat-based bioreactor [6]

Recently, the representatives of the NOVA HUSKY Research Corporation described the design and operation of a new type of bioreactor based on the use of one of the NOVA HUSKY products, an oleophilic peat termed "Oclansorb". This is a highly porous insoluble material consisting of tunnels and channels with a very high surface area. The concept of the bioreactor is that the oil-degrading consortia can be immobilized within the inert Oclansorb matrix. Because Oclansorb is hydrophobic it attracts the hydrocarbon substrate and generates a very large interface for contact between microbes and oil. This in turn should accelerate the rate of biodegradation of the hydrocarbon substrate. The bioreactor consists of a series of interconnected boxes lined along the bottom with the Oclansorb, over which sludges or tank bottoms are layered. Air is pumped into the bioreactor through a series of tubes and the whole system is covered with black plastic sheeting to allow the reactor to warm up and function at a temperature approaching 30°C even in the winter. The reactor has been scaled to a level where several hundred kilos can be processed. The complete cycle, which lasts about 5 weeks, leads to about 70 percent biodegradation of the input sludge, with the expected accumulation of inert asphaltenic materials. Interestingly, the reactor need be inoculated only once, since the hydrocarbon-degrading organisms apparently adhere to the surface of the channels and tunnels within the peat as they grow, and are not washed out during the cleaning or addition of fresh sludge. No accumulation of toxic products or heavy metals was found. No information was provided

[6] *This reactor was described by Dr. Tom Jack of the NOVA HUSKY Research Corporation in his talk at the TDC Workshop on Bioremediation in Montreal.*

on the characteristics of the oil-degrading consortium either before or after the cycle, nor was any evidence presented concerning the chemical composition of the nutrients added to the bioreactor.

The physical structure of the Oclansorb suggests that it may have some potential advantage in stimulating bioremediation systems along coastal regions. In fact, Lee and Levy (39) have studied the effectiveness of chemical surfactants in stimulating biodegradation of oil pollution in a variety of beach environments.

4.1.3 Open vs closed systems: a summary

Closed systems offer a number of advantages over the open environment (see Table 4). One advantage is that because nutrient wash-out does not occur, sources of nitrogen and phosphorous can be used that are much cheaper than the oleophilic fertilizers. Moreover, the enclosed environment offers better conditions for controlling the process, although retrofitting is required. Again, because of the closed environment, seeding with more efficient microbial consortia may be carried out. For closed systems batchwise biodegradation processes are more easily modelled and simulated in the laboratory, making their scale-up simpler. An additional advantage lies in the ability to clean containers with odd shapes and surfaces that are difficult to reach. Not only are tanks cleaned, but also they can be rendered gas-free. Finally, in a situation where a number of tanks need to be cleaned, a portion of a previously remediated tank may serve as an inoculum for bioremediation in a second tank. This kind of cycling may lend itself to certain continuous processes.

Bioremediation in closed tanks has a number of disadvantages. The tanks must be retrofitted to contain aeration systems, which may be neither simple nor inexpensive. In addition, temperature must be maintained. Tanks can be polluted with oils and sludges, many of which are the products of sedimentation and settling over a number of years. Therefore, prior laboratory work is needed to establish specific conditions, isolate specific consortia, and model the system prior to scale-up. The sensitivity of the biodegradation process to high concentrations of petroleum products may require prior desludging before starting the bioremediation (in the clean-up of storage tanks in tank farms, for example). Another disadvantage may lie in the potential corrosive effects associated with acid production in a sea-water environment. Although this has yet to be demonstrated, the possibility of corrosion should be studied. Finally, bioremediation in storage tanks either on land or at sea on oceangoing vessels may generate large volumes of process water that must be disposed of. While the remediation process itself is likely to render that water suitable for disposal, local regulatory agencies will have to be satisfied before treated water is deemed suitable for dumping or for use in irrigation systems.

Table 4 – Bioremediation in closed systems

ADVANTAGES	DISADVANTAGES
• LOWER COSTS FOR NUTRIENTS • BETTER CONDITIONS FOR CONTROLLING THE PROCESS • SEEDING IS POSSIBLE • LABORATORY MODELLING, SCALE-UP AND SIMULATIONS ARE EASIER • TANKS OF ODD SHAPES AND SIZES CAN BE CLEANED AND RENDERED GAS-FREE WITHOUT HIGH LABOUR COSTS • A NUMBER OF TANKS MIGHT BE ABLE TO BE CLEANED DURING THE SAME CLEANING OPERATION	• RETROFITTING MAY BE DIFFICULT AND EXPENSIVE • BIOREMEDIATION STILL NOT AN "OFF-THE-SHELF" TECHNOLOGY – LABORATORY WORK REQUIRED FOR SPECIFIC SLUDGES AND OILS IN TANKS • FULL TANKS MAY HAVE TO UNDERGO DESLUDGING PRIOR TO STARTING THE BIOREMEDIATION • BIODEGRADATION PROCESS MAY ENHANCE THE PROCESS OF CORROSION • LARGE VOLUMES OF PROCESS WATER MAY BE GENERATED

4.2 Fuel up-grading and gaseous emissions

Gaseous emissions from engines and boilers in the form of sulphur oxides (SO_x), nitrogen oxides (NO_x), and volatile organic compounds (VOCs) constitute major sources of environmental air pollution. Mechanical and chemical scrubbing systems from exhaust gases can be both difficult and expensive. At least three different biotechnological approaches to this problem have been considered: the use of micro-organisms in the selective removal of organically bound sulphur and/or nitrogen from crude or fuel oils prior to burning; the use of microbial products (biosurfactants) to stabilize oil-in-water or water-in-oil emulsions, which can then serve as more "environmentally friendly" fuels; and the use of microbes to detoxify VOCs. All three technologies are in their infancy and will therefore be presented only briefly. However, should any or all of them become viable technologies, the implications should be quite far reaching.

4.2.1 Desulphurization and denitrification (40-42)

Oils contain both organic and inorganic forms of sulphur and nitrogen. The latter are usually present as sulfides, nitrates and nitrites, and inorganic amine compounds. These

inorganic salts are usually present in very low amounts and technology is available for their removal. The heterocyclic sulphur and nitrogen-containing compounds such as the benzyl and di-benzyl thiophene derivatives contain covalently bound S and N very tightly bound to the oil. Although chemical technology is available to scrub the oxidized sulphur-containing gases from exhaust fumes, the technique is very expensive. Biotreatment by micro-organisms may represent one of the few ways to remove such atoms without destroying the integrity of the oil itself. One approach taken has been to try to utilize model compounds normally present in oil as major sources of organic sulphur (dibenzyl or benzyl thiophene have been tried), and to select organisms from nature that can utilize this material as a sulphur-containing nutrient, but cannot grow on oil as a carbon and energy source. Activities of this kind have recently been described and are currently being evaluated. A second approach involves isolating organisms that can assimilate and utilize organic sulphur or nitrogen from oil and can also degrade the hydrocarbons. The suggestion is to isolate the genes responsible for organic sulphur degradation, and to transfer them using cloning and genetic engineering technology to a second organism that cannot grow on oil, but will inherit the property of sulphur oxidation. The approach looks promising since, in the cases where sulphur-oxidizing bacteria have been isolated, the genes conferring the property appear to be localized on extrachromosomal genetic elements termed plasmids. Plasmids may be useful in mediating the gene transfer. One difficulty is that such genetically engineered organisms will also need to be able to interact physically with the oil. In principle, these obstacles can be overcome, but the research is only beginning.

4.2.2 Use of biosurfactants to form and stabilize oil-water emulsions

It is well known that the formation of homogeneous emulsions of water in oil allows for the efficient combustion of fuels. Elf-Aquitaine developed technology for the mechanical generation of such emulsions just prior to injection into the combustion chamber. The idea is that in the presence of up to 30 percent water, the water is vaporized at a much lower temperature and the oil is combusted as small droplets at a much higher surface area. The efficiency of combustion is presumably increased. Conventional mechanical emulsifiers have suffered maintenance and performance problems, so that the emulsions formed were insufficiently homogeneous prior to injection.

One approach to the problem is the use of chemical formulations to make and stabilize emulsions of appropriate hydrocarbon content that are sufficiently homogeneous and stable to be injected and burned efficiently.

As mentioned previously, oil-degrading micro-organisms frequently produce extracellular products such as biosurfactants and biopolymers. One such product, termed emulsan, was discovered in the author's laboratory (42). Emulsan is a high molecular weight biopolymer with properties that cause it to go directly to an oil-water interface and to form a film around the surface of an oil droplet. The polymer orients itself around the droplet in such a way that two droplets coming together repel each other and oil in the emulsion formed does not coalesce, since the droplets remain freely suspended in the water as cream rather than as an intact layer of oil. Such emulsan-stabilized oil creams (oil-in-water emulsions) can contain up to 30 percent water and still remain stable for years. The technology involves using formulations for the emulsification in water of very heavy oils (including vacuum residuals from refineries), rendering them pumpable and burnable as boiler fuel. The feasibility of the approach has been tested and scaled with hundreds of barrels of stabilized oil-in-water emulsions in industrial power plants. Moreover, since the resulting emulsions are oil-in-water, they can be pumped regardless of the viscosity of the hydrocarbon emulsified, even at room temperature. This technology, developed by Petroferm, U.S.A., has been applied in the following way: storage tanks were cleaned with biosurfactant-stabilized formulations, and the resulting emulsions were washed to remove bottom sediments and salts, after which the clean emulsions were pumped to boilers where they were shown to burn in a way indistinguishable from light high quality No. 2 fuel oil. The NO_x, measured as NO_2, was found to be decreased by over 15%. The corollary to this application is that very viscous oil-in-water emulsions can be stabilized using suitable formulations and pumped very long distances through pipelines without the need for heating. The cost-effectiveness of the technology depends on the cost per barrel of oil compared with the cost to produce polymer by fermentation (which is very expensive). The success of the biosurfactant-stabilized formulations has prompted a search for other cheaper chemicals that can replace the more expensive bioproduct. Such formulations have been described and used in a waste treatment program in which a chemical lagoon consisting of organo-chemical waste was cleaned by emulsification. Oil-water emulsions were formed using the emulsifying formulation by pumping for several cycles. They were subsequently burned in a boiler system with the concomitant recovery of energy from the emulsified chemical waste product. The cost-effectiveness of this approach may lie in the ability to take credits on the energy savings from the combustion of the emulsion.

4.2.3 Bioremediation of VOCs

Fuels contain significant quantities of volatile materials, such as the aromatic hydrocarbons, benzene, and toluene, as well as other compounds emitted during the combustion of petroleum products. Recently, organisms have been isolated capable of degrading

these materials as well as other VOCs. The hope is that such organisms will offer a means of bioremediation of these noxious pollutants. The technology will apparently involve the immobilization of VOC-degrading organisms onto biofilters that can then absorb the noxious materials, bringing about effective remediation. The work is only now at its beginning stages. If successful, the technology of VOC-degrading biofilters should be applicable to exhaust and flue gases from ships, as well as to industrial environments.

4.3 Microbial corrosion (44)

Corrosion of steel structures both on land and on ships is a major problem worldwide. For example, it has been estimated that each year metal corrosion (marine and non-marine) costs the U.S. nearly $200 billion in operational problems and economic loss. In addition, the U.S. Navy estimates its annual economic loss due to corrosion failures at over $5 billion. In this regard, bacteria are thought to be responsible for corrosion damage to over 75 percent of the productive oil wells in the U.S., as well as for over half the failures of buried pipelines and cables (44).

4.3.1 Biological corrosion phenomena (45)

Bacteria and other marine organisms attach to metal surfaces with such efficiency that within a week or so following immersion, metal surfaces are found colonized by bacteria, algae, and protozoa. Microbial involvement in a wide range of corrosion phenomena has been recognized (see Table 5). The major activity is thought to be the generation of both acids and sulphides by corrosive bacteria. Two kinds of organisms are generally isolated from corrosion sites, although their precise role(s) in corrosion enhancement has not been proven: sulphate-reducing bacteria (SRBs) and acid-producing bacteria (APBs). Of these, SRBs are considered the major corrosion-enhancing organisms. One typical representative of SRBs is a bacterium termed *Desulfovibrio* which grows in the absence of oxygen. *Desulfovibrio* carries out a reaction based on the oxidation of hydrogen gas and the concomitant reduction of sulphate to form hydrogen sulphide. The model recently proposed to account for the role of sulphides in corrosion is that the sulphides scavenge electrons from elemental Fe^0 to Fe^{+2}. The rapid microbial growth produces large quantities of the iron sulphides, thereby stimulating corrosion. Other micro-organisms implicated in the corrosion process include methane-producing organisms, nitrate reducers, and certain thermophiles (bacteria living at high temperatures). The action of SRBs is increasingly being viewed as just one of many possible biological influences on corrosion reactions (see Table 5).

Table 5– Biological corrosion phenomena

- DIRECT INFLUENCE ON THE RATE OF ANODIC AND CATHODIC REACTIONS
- GENERATION OF CORROSIVE METABOLITES SUCH AS SULPHIDES AND ACIDS LEADING TO THE CREATION OF A CORROSIVE ENVIRONMENT
- CHANGE OF SURFACE METAL FILM RESISTANCE BY MICROBIAL METABOLISM OR METABOLITE PRODUCTION
- CREATION OF ELECTROLYTIC CELLS ON THE METAL SURFACE BY THE UNEVEN DISTRIBUTION OF MICROBIAL COLONIES OR EXTRACELLULAR "SLIMES" ON THE SURFACE
- BIODEGRADATION OF CORROSION INHIBITORS AND PROTECTIVE PAINTS AND COATINGS

Most anticorrosion treatments include cathodic protection systems as well as coatings that serve as barriers to prevent oxidation of the iron. Moreover, paints are treated with biocides, which allegedly kill off the biofouling organisms and corrosion-causing SRBs and APBs. Most biocides are themselves highly toxic materials, and many have been shown to be ineffective. In ship piping systems the imposition of an impressed surface current can be used to inhibit the oxidation of the iron in the steel.

Prevention of biological corrosion will require a detailed knowledge of the organisms involved, since new design criteria for corrosion inhibitors, biocides, and appropriate alloys will depend on the specific microbial processes likely to occur.

4.3.2 Corrosion diagnostics

As a first step in unravelling this system, newer and more modern techniques, including molecular biological approaches, have been designed to differentiate between the various bacteria isolated from specific sites of corrosion in pipelines and other steel structures. Amazingly, there are at least 50 different corrosion-inducing SRBs, none of which is similar to the other except for the ability to reduce sulphate to make sulphide (46). In fact, a new patent application is designed to use a system of detection to diagnose the potential for corrosion in some sort of early warning system. The problem is similar to the problem of early detection of dental plaque formation by searching for characteristic micro-organisms.

This general approach of using sophisticated techniques to dissect a microbial population for organisms whose physiological characteristics may be either damaging or beneficial in a specific environment has become an extremely important concept in environmental microbiology (47).

4.4 Bioremediation in airport environments

The activities in airports are also of major environmental concern. Two such activities in Canada are of particular importance: the widespread use of deicing fluids on wings of airplanes in winter; and the extensive use of hydrocarbons in the generation of model fires for firemen's training activities (FTAs). In both cases large amounts of environmental pollutants are employed. Their accumulation and subsequent disposal becomes significant. In both cases bioremediation may be a useful technology for environmental maintenance. The two areas of application are described briefly below.

4.4.1 Bioremediation of deicing fluids (48, 49)

The deicing of wing surfaces becomes a major activity in northern airports in winter. The process usually involves the use of very large quantities of a formulation comprising a glycol/water solution together with a wetting agent, an emulsifier, a corrosion inhibitor, and some colouring dye. The deicing glycols can be mixtures of ethylene, propylene, or butylene glycols in various ratios. Future deicing fluids may be polymeric derivatives of the glycols, which exhibit better performance, but cannot be recycled. In the absence of any treatment, significant quantities of the material mixes with surface waters and runs off the apron into storm drains and subsequently into nearby streams. It has been estimated that over 2000 t of deicing fluids are used at Montreal's Dorval airport each year. Although the material has been shown not to accumulate in plant or animal tissues, it does represent a serious load of excess carbon in the environment. Current environmental legislation requires that the deicing fluids no longer be allowed to run off. They must be collected at the terminal, where they are initially applied, and again at the end of the runway after they are applied a second time to prevent ice formation as airplanes wait their turn for take-off. The disposal of this accumulating deicing material presents a major environmental problem. Although most of the materials in current use can theoretically be recycled, this is only cost-effective when the glycol concentration in the water is above 15 percent.

Bioremediation has been suggested as a viable alternative, given the high rates of biodegradation under ideal conditions of temperature and input pollutant concentration (< 1 percent). Two major problems appear to affect the rate of biodegradation of deicing fluids: bacterial membranes are highly sensitive to the glycols, and at concentrations

above 2-3 percent the glycol substrates actually kill the degrading organisms; and the rates of degradation are highly sensitive to temperature. Since the glycols may be stored at concentrations of 15 percent, the materials would have to be diluted by a factor of between 10 and 20 with process water to lower the effective concentration to a point where it would not affect the degrading organisms. The temperature limitations have been overcome through the design of a new bioreactor patented through Energy, Mines and Resources Canada. In temperature-controlled environments and low concentrations, rates of degradation are very high. At Dorval airport, an activated sludge system for remediation of deicing fluids is being contemplated. Here the problem once again is temperature and concentration.

One approach to the temperature difficulty may be to use cold tolerant organisms capable of degrading deicing fluids. The author identified the ice tank at the Institute for Marine Dynamics in St. John's, Newfoundland, as one potential source of such organisms. Deicing fluids are employed in this tank to control the rate of ice formation. The refrigeration unit uses ammonia, which is leaking enough to make its odour easily detected. There were clear signs of biofouling in the tank, although the author's guide was quite convincing as to its cleanliness. The hypothesis is that such tanks contain microorganisms capable of utilizing the deicing fluid as a source of carbon and the leaking ammonia as a source of nitrogen. Such a tank may be an ideal source of low temperature "deicing" organisms.

4.4.2 Firemen's Training Activities (FTAs)

Many airports routinely conduct on-the-job training exercises for firemen. Some exercises involve the use of gasoline to generate a fire that the trainees extinguish. The efficiency with which the fires are extinguished usually leads to the combustion of only about 60 percent of the gasoline hydrocarbons, with the remaining 40 percent draining into the St. Lawrence River. Between the two Montreal airports of Mirabel and Dorval, the non-combusted hydrocarbons can amount to over 20 000 gallons per year. The magnitude of the problem is probably about 500 000 gallons worldwide.

Current plans call for the excavation of a pit and the use of *in situ* bioremediation to remove the 100 ppm oil and grease normally present in the water. The remediation could be conducted in a second tank connected to the gravity separator. The solution is quite practical since the FTAs are concentrated into a few months of the year. The remaining period could be used to carry out the bioremediation of the accumulated non-combusted hydrocarbon.

5. STATE OF THE ART IN CANADA

As stated in the Introduction one of the author's objectives as TDC's Visiting Expert was to determine the status of bioremediation R&D throughout Canada. To accomplish this, visits were made to various government, academic, and industrial institutions and laboratories over a one-month period. In addition, the author was invited to speak both formally and informally at several institutions, where he met with various individuals involved in different aspects of pollution research and development. Finally, the author organized and chaired a Workshop on Bioremediation of Marine Oil Pollution, which was held in Montreal at the beginning of October 1991. This gave him the opportunity of meeting many of the major players from a variety of regions throughout Canada.

5.1 Technical areas of interest

A list of technical areas of potential interest is provided in Table 6. This division into sub-topics is highly subjective and reflects the author's own viewpoint of the breadth as well as the scope of biotechnology related to bioremediation of oil pollution. Others might have chosen a different emphasis. Several of the topics would appear to have a direct impact on bioremediation technology, including: microbiology, analytical chemistry, product testing and evaluation, field tests, environmental effects of pollution, management of field experiments, emergency spill clean-up, and various aspects of environmental consulting. Such diverse subjects as biochemical engineering and bioreactors, biofouling and biocorrosion, molecular biology and chemistry, bioremediation of deicing fluids, and desulphurization and fuel upgrading were included because they are highly relevant transport-related problems; they are considered to be major areas for potential applications of environmental biotechnology (see Sections 3 and 4); and they may provide short-term R&D projects of interest to Transport Canada in general and TDC in particular (see Section 7).

5.2 Expertise – list of experts

During the course of his stay in Canada, the author met with professional researchers, academicians, engineers, and technologists, many of whom have demonstrated expertise in one or more of the areas listed in Table 6. These individuals are listed in Table 7. Several the author knew personally before coming to Canada; others were known to him through their publications in the professional literature. In some cases, the expert may be affiliated with an institution in which a number of other experts are working on similar or related projects. The names of these people were included as well, unless the author was not informed about their activities. Finally, some other scientists expressed

Table 6 — Technical areas of interest

- MICROBIOLOGY: PETROLEUM, ENVIRONMENTAL, PHYSIOLOGY
- BIOCHEMICAL ENGINEERING, BIOREACTORS
- ANALYTICAL CHEMISTRY
- PRODUCT TESTING AND EVALUATION
- BIOFOULING AND BIOCORROSION
- MOLECULAR BIOLOGY AND CHEMISTRY
- FIELD TESTS: MICROBIOLOGY, FATE OF OIL, CHEMISTRY
- ENVIRONMENTAL EFFECTS OF POLLUTION
- FIELD EXPERIMENT MANAGEMENT
- SPILL CLEAN-UP
- BIOREMEDIATION OF DEICING FLUIDS
- DESULPHURIZATION AND FUEL UPGRADING

an interest in working in environmental issues. The author felt that on the basis of their previous accomplishments in other directions they were likely to make contributions to various bioremediation programs as well.

This list is far from comprehensive. Probably twice as many experts in various areas are working in Canada, but the author neither met nor knew of them. The reader is referred to the Directory of Canadian Marine Oilspill Specialists (50) for an extensive listing. There are notable omissions in this listing, including many experts in allied technologies and even some microbiologists. Significantly, one of the premier analytical laboratories in Canada is not represented in the Directory.

Table 7 — List of experts

EXPERT	AFFILIATION
Dr. J. BUNCH	Department of Fisheries and Oceans, Ottawa
Dr. J.-GUY BISAILLON	Armand Frappier Institute, UQAM, Montreal
Dr. D. COOPER	Department of Chemical Engineering, McGill University, Montreal
Dr. J.W. COSTERTON	Department of Microbiology, University of Calgary, Calgary
Dr. W. CRETNEY	Institute of Ocean Sciences, Patricia Bay, Victoria
Mr. P. DEVENIS	Canadian Petroleum Association, Calgary
Mr. D. DICKENS	D.F. Dickens Associates Ltd.
Dr. P. FEDORAK	Department of Microbiology, University of Alberta, Edmonton
Mr. M. FINGAS	Emergency Sciences Division, Environment Canada, River Road Labs., Ottawa
Dr. M. FRANCIS	NOVA HUSKY Research Corporation, Calgary
Dr. R. GOODMAN	Esso, Calgary
Dr. D. GOULD	CANMET, Ottawa
Dr. C. GREER	Biotechnology Research Institute, Montreal
Ms. C. HOGAN	CANMET, Ottawa
Dr. J. INGRAHAM	Department of Microbiology, McGill University, MacDonald Campus, Montreal
Dr. T. JACK	NOVA HUSKY Research Corporation, Calgary
Dr. W. KAY	Microtek, Research and Development, Victoria
Dr. V.I. LAKSHMANAN	Ortech International, Mississauga, Ontario
Dr. K. LEE	Institut Maurice LaMontagne, Mont-Joli, Quebec
Mr. S. LÉGER	Monenco, Montreal
Dr. E. LEVY	Bedford Institute, Dartmouth, Nova Scotia
Dr. A. MACLEOD	CANMET, Ottawa

Table 7 — List of experts (cont'd)

EXPERT	AFFILIATION
Dr. R. McCREADY	CARE International, Calgary
Dr. A. McCULLOCH	Institute for Marine Biosciences, Halifax
Dr. M. MOO-YOUNG	Department of Biochemical Engineering, University of Waterloo
Dr. R. NEUFELD	Department of Chemical Engineering, McGill University, Montreal
Dr. E. NEWMAN	Department of Biology, Concordia University, Montreal
Dr. J. PAYNE	Department of Fisheries and Oceans, St. John's, Newfoundland
Dr. B. RAIMY	Department of Microbiology, UBC, Vancouver
Dr. V. RHAMATULA	Department of Biochemistry, Memorial University, St. John's, Newfoundland
Capt. W. RYAN	CCG, Newfoundland Region
Mr. Y. SABEH	Transport Canada, Dorval Airport, Montreal
Dr. B. SANKEY	Esso Resources Canada, Calgary
Mr. G. SERGY	Environment Canada, Edmonton
Mr. A. SILVA	Petro-Canada, Calgary
Dr. G. SIM	Institute for Marine Biosciences, Halifax
Dr. G. SPIEGELMAN	Department of Microbiology, UBC, Vancouver
Dr. L. STEHMEIER	NOVA HUSKY Research Corporation, Calgary
Dr. M. SYLVESTRE	Institut national de recherches scientifiques (INRS), UQAM, Pointe-Claire
Dr. J. VANDERMEULEN	Bedford Institute, Dartmouth
Dr. G. VOORDOUW	Department of Biochemistry, University of Calgary
Dr. D. WESTLAKE	WestTec Microbes Ltd., Victoria
Dr. C. WYNDHAM	Department of Biology, Carleton University

5.3 A TDC directory

Table 8 presents a matrix directory linking particular experts with their areas of expertise. Frequently, the individual may have experience and accomplishments in a wide variety of fields (e.g., Fingas or Jack). In part this may be because the expert has a number of workers and laboratories under his direction. In other instances, the expert may have taken part in projects that included several of the activities listed in the TDC Directory. For example, Dr. Ron McCready may be a microbial physiologist by training, but his work at CANMET involved the design and even patenting of a bioreactor for bioremediation.

One of the smallest groups listed is in the area of Environmental Consulting. For this group, the author listed only those individuals he met personally. In fact, this is likely to be one of the largest groups in Canada. Most of the environmental consultants in bioremediation of oil spills are listed in the Directory of Canadian Marine Oilspill Specialists (50).

Since the projects managed by TDC in the area of bioremediation or other environmental biotechnologies are likely to be multidisciplinary in nature, the matrix can be used to quickly formulate small groups of experts contributing to an overall project.

Table 8 can be readily upgraded and modified as TDC personnel develop new contacts and associations. For example, environmental engineering and biochemical engineering could be separated into two different categories. Moreover, spill clean-up technology could be divided between marine and terrestrial spills. Other modifications could include the addition of new categories such as: chemical spills (both on land and at sea); government administrative and regulatory bodies; private companies marketing microbial consortia for bioremediation; and production of new microbial products. Such topics are beyond the scope of this study. Moreover, the upgrading of Table 8 is best done by TDC personnel themselves during the course of their involvement in specific projects. In addition, as TDC officers gain experience in different projects, they can add or subtract names from the matrix and include a means of evaluating various players.

By and large the author found Canadian expertise to be at a par with the best known groups in the world. For example, Environment Canada is one of the world leaders in developing protocols, testing products, and standardizing procedures for evaluation of both efficacy and toxicity. Moreover, because of its close connections with the U.S. EPA it has accumulated unique experience in management of field experiments and large-scale trials. This has placed Canada in a unique position, particularly in North America.

Table 8 – Directory of selected Canadian experts and fields of expertise

EXPERT	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
BUNCH	•						•							
BISAILLON	•						•							
COOPER		•												
COSTERTON	•				•		•							
CRETNEY			•					•		•				
DEVENIS										•				
DICKENS														•
FEDORAK	•				•								•	
FINGAS			•	•			•	•	•	•				
FRANCIS	•	•												
GOODMAN										•				•
GOULD	•	•			•							•		
GREER	•					•								
HOGAN			•										•	
INGRAHAM	•													
JACK	•	•		•	•		•			•			•	
KAY	•					•							•	
LAKSHMANAN					•									

Table 8 – Directory of selected Canadian experts and fields of expertise (cont'd)

EXPERT	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
LEE	•						•							
LÉGER		•												•
LEVY			•					•						
MACLEOD					•									
McCREADY		•			•							•		
McCULLOCH			•											
MOO-YOUNG		•												
NEUFELD		•												
NEWMAN	•					•								
PAYNE									•					
RAIMY	•	•												
RHAMATULA									•					
RYAN											•			
SABEH		•										•		

I *PETROLEUM MICROBIOLOGY, ENVIRONMENTAL MICROBIOLOGY, MICROBIAL PHYSIOLOGY*
II *ENVIRONMENTAL ENGINEERING, BIOREACTOR*
III *ANALYTICAL CHEMISTRY*
IV *PRODUCT TESTING AND EVALUATION*
V *BIOFOULING AND BIOLOGICAL CORROSION*
VI *MOLECULAR BIOLOGY*
VII *FIELD TESTS – MICROBIOLOGY*
VIII *FIELD TESTS – FATE OF OIL IN ENVIRONMENT*
IX *ENVIRONMENTAL EFFECTS OF OIL POLLUTION*
X *FIELD EXPERIMENT MANAGEMENT*
XI *LOGISTIC SUPPORT FOR EXPERIMENTAL SPILLS*
XII *BIOREMEDIATION OF DEICING FLUIDS*
XIII *DESULPHURIZATION AND OIL UPGRADING*
XIV *ENVIRONMENTAL CONSULTING*

Table 8 – Directory of selected Canadian experts and fields of expertise (cont'd)

EXPERT	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
SANKEY													•	
SERGY									•	•				
SILVA														•
SIM			•											
SPIEGELMAN						•								
STEHMEIER		•					•							
SYLVESTRE						•								
VANDERMEULEN								•	•	•				
VOORDOUW						•								
WESTLAKE	•						•					•	•	
WYNDHAM	•					•								

I *PETROLEUM MICROBIOLOGY, ENVIRONMENTAL MICROBIOLOGY, MICROBIAL PHYSIOLOGY*
II *ENVIRONMENTAL ENGINEERING, BIOREACTOR*
III *ANALYTICAL CHEMISTRY*
IV *PRODUCT TESTING AND EVALUATION*
V *BIOFOULING AND BIOLOGICAL CORROSION*
VI *MOLECULAR BIOLOGY*
VII *FIELD TESTS – MICROBIOLOGY*
VIII *FIELD TESTS – FATE OF OIL IN ENVIRONMENT*
IX *ENVIRONMENTAL EFFECTS OF OIL POLLUTION*
X *FIELD EXPERIMENT MANAGEMENT*
XI *LOGISTIC SUPPORT FOR EXPERIMENTAL SPILLS*
XII *BIOREMEDIATION OF DEICING FLUIDS*
XIII *DESULFURIZATION AND OIL UPGRADING*
XIV *ENVIRONMENTAL CONSULTING*

The author's impression is that this leadership will probably continue. The author found that the equipment in most laboratories was state of the art. The IMB laboratories of the NRC in Halifax are of exceptional quality. Very few places in the world have this level of expertise and facilities. The author also found the facilities and personnel at the NOVA HUSKY Research Institute in Calgary to be very impressive. Similarly, the Fisheries and Oceans labs in Dartmouth, St. John's, and Victoria appeared to be of the highest calibre. Many of the people interviewed by the author were somewhat troubled by the lack of communication and what appeared to them to be a lack of coordination of large-scale research programs. Frequently, the author found himself in the awkward position of informing one Canadian group working in a particular field about the efforts and achievements of another group working in the same area, when neither group was even aware of the other's efforts. Moreover, the author encountered highly skilled researchers who could make a significant contribution to various areas of petroleum microbiology if they had an opportunity. Better coordination and dissemination of such opportunities were among the suggestions made by participants in the TDC Workshop.

In Section 7, the author recommends a number of programs and suggests a limited but coordinated route to develop these efforts. The programs are designed to utilize Canadian expertise to develop transport-related technologies and applications. In the author's opinion, the success of any of these programs will thrust Canada into the forefront in this area. Achievement of this goal will depend not only on technical expertise, but also on expert coordination and project management.

6. WORKSHOP OVERVIEW

6.1 Program and format

On 3 October 1991 a TDC Workshop on Bioremediation of Marine Oil Pollution was held in Montreal. The goals of the Workshop were:

- to bring together the major players in the field from all sectors, including academia, industry, government research centres, regulatory agencies, and coordinating bodies, to consider the key issues in bioremediation of oil pollution;
- to evaluate bioremediation technology in terms of past experience, outstanding experimental and developmental questions, efficacy, and applicability. "Does it work? If so, how well and under what conditions?";
- to consider models for future field tests in specific environments, with a view to establishing a priority list.

6.1.1 Subjects covered

The program of the Workshop, shown in Appendix A, was designed around three major areas: Microbiology, Field Experiments, and Allied Technologies. It also took into account the Arctic system, one of the unique features of the Canadian scene. The keynote address was delivered by one of the pre-eminent scientists in bioremediation, Dr. Ronald Atlas. The major participants in the Workshop are listed in Appendix B, together with their affiliations and biographies. Note that in the October 1991 issue of *Scientific American* on marine oil spills, Dr. Atlas and Mr. Fingas are frequently quoted, and the work of Dr. Lee is frequently cited. Dr. Westlake and Dr. Atlas were members of the initial EPA committee that studied the problem and made recommendations for the bioremediation experiment in Alaska.

6.1.2 Format

The Workshop was divided into three sessions. During the morning session formal presentations were given by Drs. Westlake, Atlas, Lee, Jack, and Mr. McCormick. After lunch, participants joined one of three independent sessions: Microbiology, Field Experiments, and Allied Technologies. Originally a fourth group was scheduled to convene under the chairmanship of Mr. Gary Sergy. However, because of his last minute cancellation, the Arctic issues were discussed in the Field Experiment Session chaired by Mr. Mervin Fingas.

All the participants reconvened for the final session of the afternoon to listen to the reports presented by the session chairmen. During that session the major issues were discussed and some priorities were attached to specific projects.

6.2 Summaries of presentations

6.2.1 Dr. Ron Atlas – Keynote lecture

Beginning with a brief historical outline, Dr. Atlas showed the development of research on the biodegradation of hydrocarbons, which began with an attempt in the 1950s and 1960s to produce single cell protein from organisms growing on hydrocarbon substrates[7]. In the 1970s researchers became aware of the potential for using biodegradation for the treatment of oil pollution problems in terrestrial environments such as refineries and other industrial environments, as well as for various ground water pollution problems. In the 80s and 90s large-scale attempts to apply bioremediation to major oil spills were made. This was primarily a North American effort, since the prevailing European opinion is to allow oil spills to degrade naturally. Dr. Atlas repeated the point that bioremediation is not only a scientific and technological subject, but also a political subject.

He next introduced some of the basic properties of bioremediation, pointing out that virtually hundreds of species of microbes are capable of hydrocarbon degradation and that different fractions are degraded at different rates. Moreover, these degrading populations are apparently strongly enriched in those environments where the organisms have been exposed to some pollution. In considering the *Amoco-Cadiz* spill, he mentioned an interesting phenomenon related to an adaptation period prior to oil breakdown. In examining the gas chromatographic profile of the residual oil, there was a "hump" rather than a flat base line in the region where the alkanes should have been. The simplest interpretation of this result is that the bacteria are converting the hydrocarbons into some form of undefined product. The phenomenon is still not understood. The degradation of oil in nature in the absence of added nutrients is only 0.001-0.3 g/m^3/day. This rate can be increased under optimal bioreactor conditions to 10-100 kg/m^3/day. Dr. Atlas also considered the various environmental factors affecting the rates of biodegradation, such as oxygen, nitrogen, phosphorous, and trace metals. Referring to his work as a PhD student

[7] *Author's note: the British Petroleum process for the harvesting of yeast protein from degradation of hydrocarbons emerged as a result of unsuccessful attempts to develop a cost-effective process for the dewaxing of fuel oils. The accumulation of large amounts of biomass suggested the possibility of using this material as a source of protein.*

with Richard Bartha, he described an approach to stimulating the growth of organisms on oil in open systems using so-called oleophilic fertilizers. The wave action and the growth of the microbes at the oil-water interface suggest that oxygen is not a limiting factor in marine oil spills. Dr. Atlas mentioned that he disagrees with the notion of seeding marine oil spills, since he doesn't believe that seed cultures can be applied in time to do any good on the open sea.

Before turning to the largest bioremediation experiment ever tried, the *Exxon-Valdez* spill, Dr. Atlas mentioned the concept of bioremediation testing – one of the key points being the need for a successful large-scale field trial to win public acceptance of the technology. Efficacy tests should be done in flasks, in microcosms in the environment, and finally in the field. At the same time, both chronic (long-term) and acute (short-term) toxicity studies should be carried out. Moreover, the added fertilizers need to be examined for their potential toxicity. The experimental measurements should include total microbial numbers, the relative proportion of hydrocarbon degraders, and the microbial activities (e.g., chemical analysis of the residues accumulating *in situ*. In terms of shoreline clean-up, if the oil moves away from the shore it is not necessary to remediate it. Finally, Dr. Atlas pointed out that it is frequently difficult to identify a reference point from which to compare efficacy. This may be due to heterogeneity of the terrain, unequal distribution of oil, or problems of drainage of pollution to the reference point area.

Turning to the *Exxon-Valdez* experiment, he provided some of the background. He mentioned that the *Exxon-Valdez* spill was not as large as many others. Moreover, the sea was calm, the weather was cooperating, and there was a relatively large population of oil-degrading micro-organisms in the region. Rock washing with hot water seemed not to be working very well, and appeared to be causing serious damage to the marine biota, such as bi-valves, in the area. Inipol, the fertilizer used, was not tested before its addition to the *Valdez* site. Although Inipol contained an oleic acid, there was no evidence that biodegradation caused the oil to move off shore into the water column. Dr. Atlas outlined major technical difficulties associated with Exxon's effort, including: the difficulty of assessing how to carry out a statistically accurate demonstration[8]; the conflict between the need to react quickly to a spill and the need for a field demonstration prior to large-scale fertilization; the need for better analytical chemical methods (pristane/phytane ratios to normal alkane degradation were found not to be applicable); the problems of physical disturbances and inability to control intrusion into the site of the experiment; and the lack of an available testing facility.

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[8] *Many of these issues were taken up by the Field Experiments discussion group and are discussed in Section 3. They will only be listed here.*

Finally, Dr. Atlas, while not as strong in his opposition to seeding as Dr. Westlake, expressed his own personal support of the use of fertilizers without seeding. He did, however, mention that if seeding does prove to be of benefit, it might be worthwhile to examine the use of ships to provide seed cultures.

6.2.2 Dr. Don Westlake – Microbiology

Dr. Westlake introduced the topic of biodegradation of oil and covered many of the points in Section 2 of this report. He provided an example of the extremely low rate of oil degradation in the absence of added nutrients: a spill in Norman Wells, where intact oil was still detectable after 35 years. He pointed out that the rate of degradation depends not only on nutrients, but also on oxygen and temperature. Turning to a consideration of the complexity of the oil substrate (containing over 2000 chemicals) he provided an example of the independence of the breakdown processes: exposing oil to an alkane degrading population of bacteria caused the accumulation of the aromatic fraction, while alkane accumulation was seen with an aromatic degrader. In the presence of the two strains, degradation of both alkane and aromatic fractions was observed. Consequently, Dr. Westlake stressed, coordinated breakdown of oil by consortia of micro-organisms is needed. He also dealt with the accumulation of so-called recalcitrant molecules such as the asphaltenes. He emphasized that, depending on the type of oil and the location of the spill, the relative amounts of accumulated asphaltenes might vary considerably. He concluded his presentation with a list of research needs, including:

- development of nutrient formulations and their applications in environmental spill conditions;
- development of an understanding of the genetics of hydrocarbon-degrading organisms for the purpose of understanding the interrelationships between laboratory strains and natural isolates;
- development of an understanding of the behaviour of organisms in natural environments in order to assess the survival of potential seed cultures.

In summarizing the relative contribution of bioremediation to spill clean-up, Dr. Westlake pointed out that once a spill occurs the order of activities should be:

- containment of the spill;
- physical/mechanical oil removal;
- bioremediation.

6.2.3 Dr. Ken Lee – Field experiments[9]

Dr. Lee described his early experiments working with microcosms (enclosed systems exposed to the environment, in which oiled sediments can be studied without washout) and analyzing microbial growth correlated with oil degradation. He made an interesting point related to natural hydrocarbon seepage, stating that oil-degrading micro-organisms have been found in areas as deep as 600 m in the Canadian Arctic. He then described experiments in Nova Scotia showing that nutrient fertilizers had to be added semi-continuously to achieve oil degradation; that the addition of commercially available strains of oil-degrading bacteria along with nutrients had no effect and, in fact, the lab strains died off within 60 days; and that agricultural fertilizers constituted better nutrient supplements that Inipol (EAP 22). In fact, it appears that the oleic acid in Inipol is the preferred carbon source of the indigenous organisms rather than the oil itself. He also described his experiments in a sand beach environment, which showed that low concentrations of a waxy crude oil (0.3 percent Terra Nova) could be degraded in a matter of days by the indigenous biota, while higher concentrations (3 percent) were much more persistent (components as light as n-C_{11} remained after 6 months). These results suggest that clean-up of crude oil spills at low concentrations may be left to nature, since natural biodegradation processes occurred rapidly. At higher oil concentrations, however, the natural biodegradation rates for oil could be accelerated by the application of fertilizers, without adverse effects. Preliminary laboratory experiments conducted at low temperatures suggested that nutrients might have some effects in Arctic environments, but biodegradation experiments at the underside of ice are needed.[10]

6.2.4 Mr. George McCormick – The Arctic system

Mr. McCormick's presentation dealt with the following topics:

- The unique characteristics of the Arctic
- Its importance to wildlife
- Sources of oil pollution
- Causes of spills
- Location of significant drilling activity in the Beaufort Sea, Arctic Islands, East Coast, and West Coast

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[9] *Dr. Lee's presentation is summarized in Section 3 and will be presented only briefly here.*

[10] *Author's note: This may be in contrast to other reports (51).*

- Characteristics of Arctic crude
- Hazards presented to oil spill clean-up
- Oil spill research

He pointed out that the Arctic represents over two-thirds of Canada's coastline, with considerable distances between communities. Access to these areas is seasonal to non-existent. Landfast ice protects the shoreline so that one can be isolated on land for periods of up to 9 months.

Among the pollution sources in the Arctic McCormick listed:

- ships (pollution from both bilges and spills)
- tank farms
- offshore drilling activity
- pipelines
- trucking

In addition, he quoted from the Brander-Smith report (2) that the cause of spills in the Arctic included 36 percent due to mechanical failure, 27 percent due to human error, and 20 percent unknown.[11] McCormick placed the problem of oil-spill clean-up procedures in perspective by outlining many of the associated hazards, including:

- wide variety of shorelines
- largely uncharted waters
- harsh climate and slow biological processes
- difficult working conditions, limited accommodations, remote air strips, poor flying weather
- difficulties in logistics
- limited spill response equipment on tankers and icebreakers

[11] *Author's note: Could these be intentional spills of bilges and/or oily ballast?*

In terms of oil-spill research, he mentioned the seven-year, $120 million, NOGAP program, ESRF programs related to oil spills and icebergs, and PERD and BREAM projects. He showed a number of figures highlighting the major sedimentary basins in the various frontier lands: West Coast Offshore; Mainland territories; MacKenzie/Beaufort Sea; Arctic Islands, Eastern Arctic; and Hudson Bay. Distributions of both offshore and onshore wells in the Mackenzie Delta-Beaufort Sea were also shown, as well as some statistics illustrating the relative decline in oil exploration. The presentation concluded with a consideration of two of the major problems likely to affect any bioremediation project: harsh weather conditions with temperature becoming a limiting factor; and the role of ice in reducing the interaction between oil and water.

6.2.5 Dr. Tom Jack – Allied technologies[12]

Dr. Jack described some of the activities in environmental biotechnology at the NOVA HUSKY Research Corporation. He pointed out that at one time NOVA HUSKY was interested in using micro-organisms for selective plugging in oil fields in order to enhance recovery. He emphasized the general observation that in very high concentrations of hydrocarbon, bioremediation simply does not work. In discussing land farming he made the interesting point that while the land-farming system may be effective, it is likely to be disallowed in Canada as in the U.S. He then outlined some of NOVA HUSKY's bioremediation problems, including various sludges, tank bottom sediments from refineries and API separator tanks, leaks from pipeline facilities, and problems related to corrosion. He discussed the approach of utilizing a material such as oleophilic peat to immobilize the oily waste substrate in order to increase the surface area of the substrate. The peat-based bioreactor is described in detail in Section 4. After showing some electron micrographs of microbial interaction with oil at the surface of oleophilic peat, Dr. Jack pointed out some of the salient features of the bioreactor, including the following:

- The peat could be reused several times.
- Although open to the atmosphere, the bioreactor was self-heating, due to the high rate of microbial metabolism.
- The reactor had to be inoculated only once – after that it was self sustaining.

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[12] *Much of the work described by Dr. Jack is presented in Section 4. Therefore, it will be only briefly summarized here.*

- The biodegradation proceeded to completion in a matter of weeks, with complete degradation of most of the sludge and the accumulation of residual asphaltenes.
- Since it consists only of a series of layers of peat, with no moving parts, etc., the reactor can be scaled up to almost any size.

Dr. Jack then turned to some of the products of bioremediation and mentioned biosurfactants as important products of oil-degrading bacteria. One of the examples he presented was from the author's own work on the use of biopolymeric surfactants to stabilize oil-water emulsions for both pipelining and oil transportation, as well as for fuel upgrading. At the author's request, Dr. Jack also showed some of the testing facilities and outlined some of the research approaches related to biocorrosion currently being pursued at NOVA HUSKY Research Corporation.

6.3 Reports of the workshop sessions

6.3.1 Microbiology – Dr. Charles Greer, Chairman

A summary of the microbiology session is presented in Figure 2. In terms of research priorities, participants felt that the interaction of microbial consortia both aerobically and anaerobically should be investigated. The group discussed a number of questions related to various microbiological projects and felt that more attention should be focussed not only on the degradation process, but also on the potential for generating toxic materials as by-products. Moreover, the group felt that there was a possibility of using microbial metabolic activities to bring about a detoxification of certain materials (i.e., chlorinated hydrocarbons and pesticides).

Genetics of micro-organisms was considered a major research area for understanding the interrelationships among various natural isolates that may catalyze the same type of chemical reaction, as well as for use as a diagnostic tool to detect the presence of specific organisms in the environment. Another objective of genetics research is to be able to engineer specific strains that could be used for bioremediation of specific chemical pollutants in those cases where consortia are not required to bring about degradation. One example might be the case of a chemical spill. Finally, the microbiology group felt that at a fundamental level, the behaviour and interactions of organisms in natural environments were only poorly understood. It was recommended, therefore, that methods be developed for extending our knowledge of bacteria and fungi, and for monitoring that knowledge.

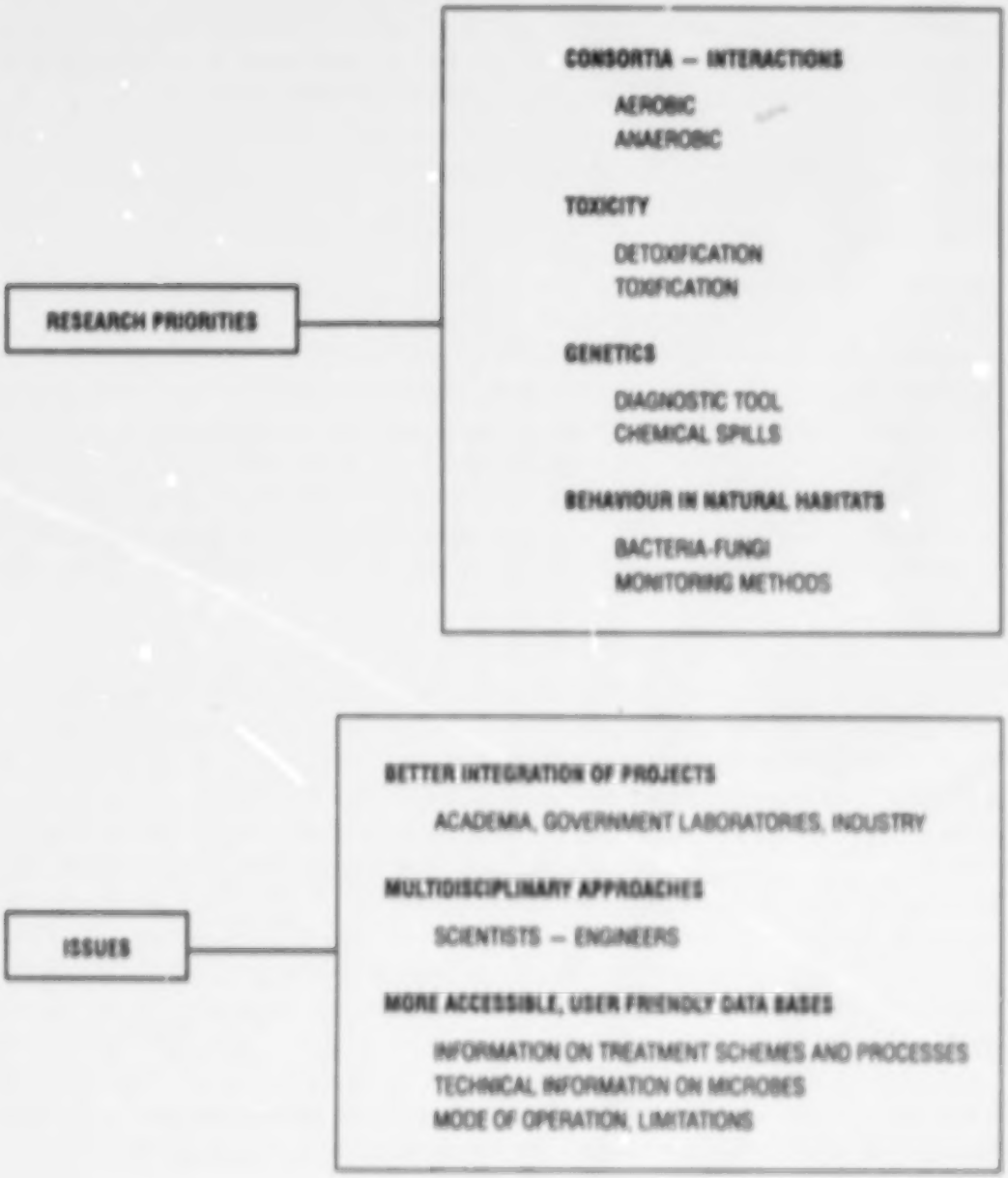

Figure 2 – Microbiology

SOURCE: Presentation by Dr Charles Greer, BRI-Montreal. TDC Workshop on Bioremediation

The microbiology group outlined a number of issues they felt needed to be addressed: better coordination of multidisciplinary projects with dialogues established between scientists and engineers; and more accessible, user-friendly data bases related to organisms, treatment schemes, processes, and other technical information.[13]

6.3.2 Field experiments -- Mr. Mervin Fingas, Chairman

Major issues (Table 9)

The field experiments group began with a consideration of the major issues outlined in Table 9. These issues were then discussed in terms of research needs. Topics included: difficulties in measurements; the need for a "best case" experiment designed to answer the question: "Does bioremediation work?" (they suggested that the research must include development of a means for evaluation of rates, extent, and limiting circumstances); the development of new fertilizers and suitable technology for their delivery; and an examination of the heterogeneity of oil substrates found in the field (tar balls, emulsions, and encapsulated oils). More political issues included questions about acceleration of field tests, obtaining permission to do experiments, Arctic issues, and bioremediation in open waters.

Interferences to good field experiments (Table 10)

The field experiments group addressed the problems of making decent measurements in a field experiment. For example, undetected physical removal of the oil from a site could easily be interpreted as being due to bioremediation. Similarly, surfactants present in the fertilizers or even generated by the microbes themselves might also cause disappearance of part of the oil, giving the impression that bioremediation caused the degredation. The group identified a number of potential weathering processes that could be enhanced, if, for example, part of the oil was complexed as an emulsion or as surfactant stabilized droplets with high surface area. As an analytical interference, the field experiments group mentioned a problem in oil measurement. For example, if oil is tied up with surfactant, it is not as easily extracted with certain routinely used solvents, and thus too low a value might be obtained, again suggesting a greater effect of bioremediation than might actually be the case. One key issue lies in the difficulties of microbial measurements. The results from the *Exxon-Valdez* experiment yielded statistically unreliable results in terms of numbers of oil-degrading micro-organisms (see Section 2).

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[13] *The author's impression was that some participants felt this information would help them to accommodate their research programs to serious applied projects and to prepare relevant and meaningful research proposals to submit to appropriate groups.*

Table 9 – Major issues – field experiments

- DIFFICULTIES IN MEASUREMENTS
- PERMISSION TO DO EXPERIMENTS
- ARCTIC ISSUES
- DOES BIOREMEDIATION WORK?
  - Need for a "best case" experiment
  - Evaluation: rates, limits, circumstances
- CONTINUE TO DEVELOP NEW FERTILIZERS AND APPLICATIONS TECHNOLOGY
- BIOREMEDIATION IN OPEN WATERS (?)
- EMULSIONS, TAR BALLS, ENCAPSULATED OIL
- ACCELERATED FIELD TESTS? – CAN'T RUSH!

SOURCE: Presentation by Mr. Marvin Fingas, Environment Canada
TDC Workshop on Bioremediation

Table 10 – Interferences to good field measurements

- UNDEFINED NOISE
- DIFFERENTIAL PHYSICAL REMOVAL
- REMOVAL BY NATURAL SURFACTANT SYSTEMS
- DIFFERENTIAL WEATHERING PROCESSES
  - Evaporation
  - Dissolution
  - Photo-oxidation
- ANALYTICAL
  - Surfactant interference
  - Partition into solvents
  - "Surrogate" measurements
- SAMPLING
- DISTURBANCES/VANDALISM
- MICROBIAL MEASUREMENTS

SOURCE: Presentation by Mr. Marvin Fingas, Environment Canada
TDC Workshop on Bioremediation

Research priorities **(Table 11)**

In their final report the field experiments group presented a list of research priorities and related these priorities to the major issues. Highest priority was given to the following areas of research:

- screening of all procedures before commencing field work;
- analytical methods; cooperative development of standardized international protocols by a standards committee;
- a "best-case field experiment designed to determine how well bioremediation works. The group felt that the microbial requirements were not well enough understood to permit the acceleration of bioremediation;
- in the area of microbiology, development and standardization of techniques and protocols for the field.

Among the medium priority subjects the group listed:

- determination of which oil substrates can be bioremediated and which (if any) cannot;
- design of an experiment to compare rates of biodegradation in the presence and absence of fertilizers.[14]

Finally, the list of recommendations dealt with some issues for which research was not required. For example, the group felt that Arctic field experiments should wait until the "best case" scenario was carried out elsewhere. Furthermore, field experiments could not be rushed, and obtaining permission to do them required political and regulatory action rather than technical activities. The group felt that a field level experiment involving bioremediation in open waters cannot be done at this time because there is currently no way to monitor efficacy. Finally, for a number of interferences listed in Table 11, better controls and experimental design are required rather than research.

[14] *Medium priority was the author's interpretation of the group's statement: "more research".*

Table 11 – Research priorities

ISSUES	SOLUTIONS	RESEARCH
SCREEN PROCEDURES BEFORE FIELD WORK		HIGHEST PRIORITY
PHYSICAL REMOVAL	CONTROLLED BY EXPERIMENTAL DESIGN	NO
SURFACTANTS	IF A NATURAL PART OF THE EXPERIMENT NEED NOT BE CONTROLLED	TEST BEFORE USE
DIFFERENTIAL WEATHERING PROCESS	CAN BE CONTROLLED BY EXPERIMENTAL DESIGN	HOW DO THESE CONTRIBUTE TO OIL REMOVAL?
ANALYTICAL	STANDARDIZE INTERNATIONAL PROTOCOL DEVELOPED COOPERATIVELY BY STUDENT COMMITTEE	HIGHEST PRIORITY
SUBSTRATE SIZE	NEED TO DETERMINE WHICH OIL SUBSTRATES REALLY WORK; CAN BE CONTROLLED	MEDIUM PRIORITY
TIME SCALE	PART OF EXPERIMENTAL DESIGN; HOW MUCH BETTER IS BIODEGRADATION THAN THE NATURAL PROCESS?	MEDIUM PRIORITY; "MORE RESEARCH"
DISTURBANCES/VANDALISM	SECURITY – MONITORING	NONE
NATURAL INHOMOGENEITY	MINIMIZE DURING EXPERIMENT	NONE
MICROBIOLOGY	DEVELOP TECHNIQUES AND PROTOCOLS FOR THE FIELD; STANDARDIZE	RESEARCH NEEDED
OTHER ISSUES	SOLUTION	RESEARCH
PRIORITY FIELD RESEARCH: HOW WELL BIOREMEDIATION WORKS, UNDER WHAT CONDITIONS, HOW TO ACCELERATE	MEASURE RATE DIFFERENTIALS, MASS BALANCES; WHAT DO THE BACTERIA WANT?	HIGHEST PRIORITY

6.3.3 Allied technologies – Dr. Ronald Neufeld, Chairman

Issues

The allied technologies group first considered a number of issues related to pollution in airports, contaminated soils, and localized pollution problems (see Figure 3). In each case individuals discussed their particular problems and/or approaches. One interesting issue raised was the dual problems of high-level and low-level contamination of soils. The low-level problem is of particular interest because currently cases of remediation at this low level are not very successful (sometimes in the order of a few ppm or even in some cases ppb for certain chemical pollutants).

Proven and emerging technologies (Figure 4)

To deal with some of these problems the allied technologies group discussed a number of proven technologies as well as a number of approaches currently being considered or about to emerge. They considered biosurfactants as emerging products of biotechnology to be used in various applications. In addition, they discussed the emergence of mobile bioreactors, whose mobility allows for bioremediation of certain pollutants to take place in different areas, and makes it possible to install small-scale bioremediation plants in different areas of sites such as refineries, airports, ships, and factories. A new technology being considered for environmental maintenance involves the use of so-called ultra-microbacteria, bacteria that are starved so that they are only about 20 percent of their regular full size. This permits the diffusibility and penetration of such organisms through channels and orifices that they might otherwise find impassable. Nutrients can then be added so that the organisms achieve full size and cannot leave the pore or channel. They then begin to produce a polysaccharide that causes plugging of the channel. One application of this "bug plug" technology is the plugging of leakages and seepage from acid mine tailings. Other technologies discussed included the use of bioproducts such as bioflocculating agents for soil conditioning, or the production of microbial products such as adsorbents or absorbents for use in various applications.

Figure 3 – Allied technologies: issues

SOURCE: Presentation by Dr. Ron Neufeld, Chem. Eng. McGill. TDC Workshop on Bioremediation

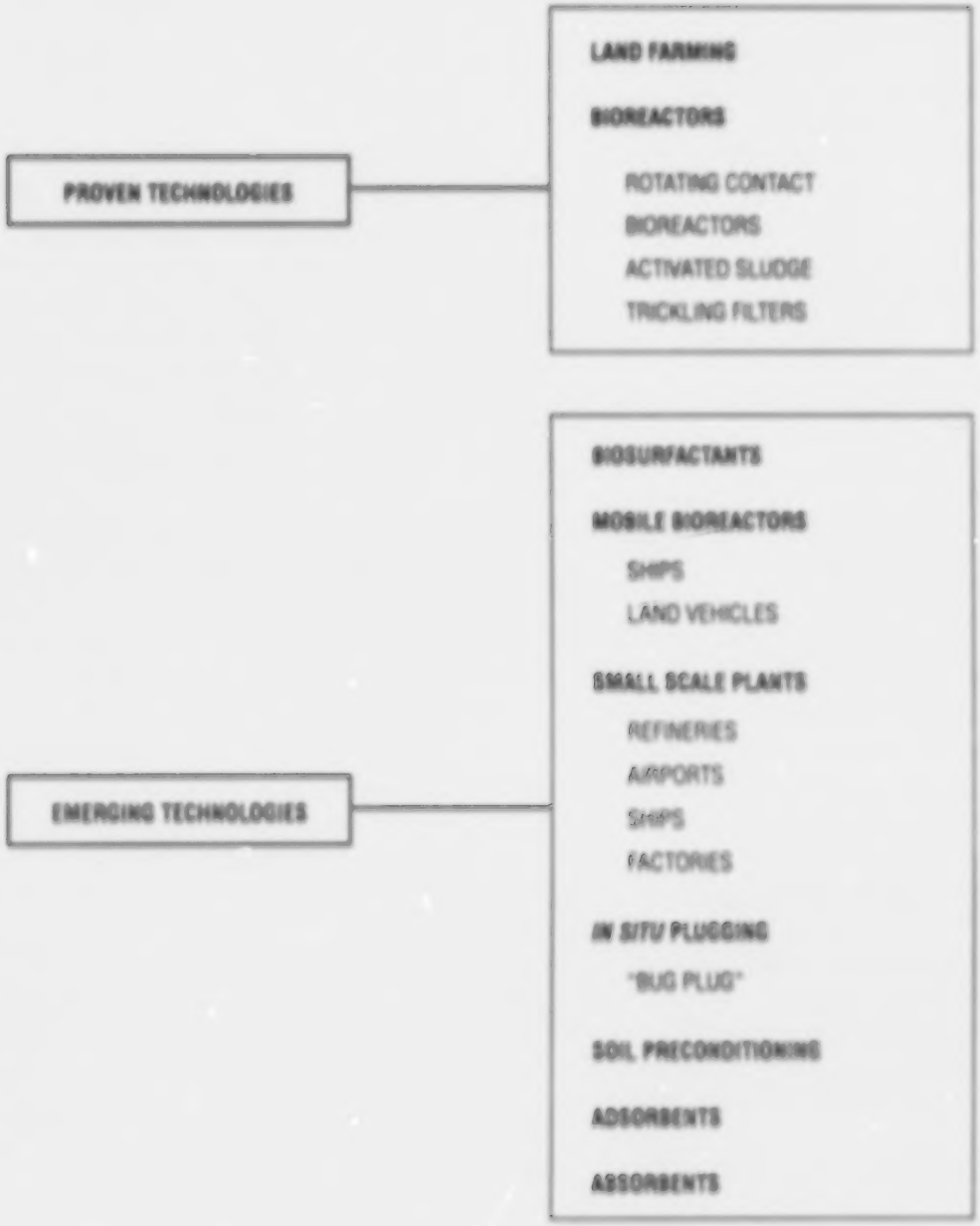

Figure 4 – Allied technologies

SOURCE: Presentation by Dr. Ron Neufeld, Chem. Eng., McGill TDC Workshop on Bioremediation

7. CONCLUSIONS AND RECOMMENDATIONS

In this report the concept of bioremediation of marine oil pollution has been extended to include a variety of biotechnologies and allied technologies that could be applied to a number of transport-related activities and problems. Two types of TDC-managed R&D programs are envisaged:

- short-term projects in which the expertise of TDC personnel in ship design and operations, marine engineering, materials, and maintenance can be brought to bear on a specific area; and
- longer-range programs in which TDC may want to consider joining with other Government agencies such as Environment Canada, the Coast Guard, Fisheries and Oceans, and Indian and Northern Affairs in participating in larger-scale field tests.

A model for one type of short-term experiment, the use of a barge as a mobile bioreactor for the treatment of off-loaded oily waste products, appears to the author to be of highest priority and is outlined in Section 7.1.1. A variation on this system, using *in situ* bioremediation to carry out extensive cleaning, de-gassing, and sludge remediation on board an oil tanker is described in Section 7.1.2.

The project described in Section 7.1.3, which involves *in situ* bioremediation of bilges, is an offshoot of the barge experiment. In the longer term, the use of the barge as a mobile bioreactor and facility for the bioremediation of bilges may be of universal significance – over 800 000 gallons of oily waste remaining in the bilge tanks of the Queen Mary were remediated by contractors on land and judged to be suitable for dumping within six weeks (17).

The remaining recommended projects are treated in less detail but their justification appears in Section 4.

7.1 Recommended programs to be executed in the short term

7.1.1 Barges as bioreactors for *in situ* bioremediation of oil and sludge

Major objectives

To establish the feasibility of using tanks on board barges as mobile *in situ* bioreactors for the bioremediation of hydrocarbon wastes such as sludges, tank washings, bottom sediments, grease, and residual oils.

Anticipated benefits

The results from this program are likely to lead to the development of a new bioremediation technology for the treatment of off-loaded sludges, grasses, sediments, and residual oil without the need to bring the pollution to the shore. The use of the barge as a mobile bioreactor "closes the system", permitting the routine remediation of oil and sludge without the need for land-based facilities. Many uses for such a system are envisioned:

- as a "floating biotreatment facility" in ports and harbours;
- in Arctic systems as a means of bioremediating sludges that would otherwise have to be stored for long periods following routine cleaning of tankers. The use of the barge would enable the biological treatment to take place on board;
- as a mobile bioreactor for the treatment of residual oil from oil- contaminated waste waters from off-shore drilling platforms. The barge facility could be used for the reduction of the residual oil to levels permitted for dumping;
- possibly, as a means of treating other more dangerous pollutants such as noxious chemicals, pesticides, or solvents.

Suggested participants

It is suggested that the project be managed by TDC along with a coordinating group from the Coast Guard. In recent conversations with the author some Coast Guard officials reacted to the concept with great enthusiasm. The technical activities should involve the participation of a microbiology group, a biochemical engineer, and a technical coordinator from the vessel itself. Technical participants could include microbiological and engineering personnel associated with the NOVA HUSKY Research Corporation, managed by Dr. Tom Jack. In addition, Dr. Donald Westlake, microbiologist, and Dr. Ronald Neufeld, biochemical engineer, are both suitable candidates. These groups have excellent laboratory and technical facilities at their disposal. For example, Dr. Westlake has access to the facilities at the University of Victoria and the University of Alberta as well as to the commercial laboratories of Microtek, Victoria. Another source of excellent biochemical engineering is the superbly equipped Biotechnology Research Institute in Montreal. NOVA HUSKY Research Corp. is quite active in a variety of bioremedial, biotechnological, and oil-related research. The microbiologist and the engineer must be able to interact and cooperate effectively with the barge personnel and to work with the coordinating and management groups.

Table 12
The use of a barge as a mobile bioreactor – a preliminary feasibility study

	PHASE I	PHASE II	PHASE III (Depends on when and where experiment held)	PHASE IV
CCG-TDC TECHNICAL STAFF	Hold series of planning meetings to: Determine time frame to fit season, etc. Consider available facilities; how many tanks available? Choose barge Determine which tank(s) Decide which oil/ sludge ("best-case scenario"?)	Meeting to complete planning Consider bench marks and determine venue		Conduct meeting with all players toward end of Phase IV: Evaluate report Plan for longer term
MICROBIOLOGIST	Laboratory experiments: Test existing consortia and isolate new strains to establish whether seeding is necessary Carry out chemical analysis to evaluate extent of biodegradation Work with bio-chemical engineer to determine optimal parameters for design details of experiment	If seeding required, continue experiments to establish scale-up conditions Stockpile and store nutrients and lab equipment for experiment	Conduct experiment Monitor biodegradation by measuring residual material Monitor growth Monitor microbial activities Isolate specific organisms Collect samples for subsequent chemical analysis	Evaluate biodegradation data and compare with chemical analysis Obtain information on make-up of microbial population before and after experiment Prepare final report

Table 12 (cont'd)
The use of a barge as a mobile bioreactor – a preliminary feasibility study

	PHASE I	PHASE II	PHASE III	PHASE IV
VESSEL COORDINATOR AND ENGINEER	Work with micro-personnel to determine optimal parameters: rates and limitations Begin to examine temperature control and air supply system for possible retrofit using steam lines, vessel compressors, etc. Provide oil and sludge samples for establishment of gasoline composition	Carry out retrofit Install air lines Temperature control Equipment Sampling apparatus, etc.	Conduct experiment Monitor tank conditions throughout experiment	Evaluate performance of equipment and work with micro-personnel to evaluate kinetics, extents, mass balance (how much carbon consumed, how much CO_2 evolution), biomass concentration, etc.
BENCHMARKS	Vessel chosen Preliminary working schedule and target date established Tanks chosen Parameters for optimal biodegradation in place Decision whether seeding necessary	Tanks are retrofitted Inocula are prepared	Preliminary data on success of experiment: Rates and extents of degradation estimated Preliminary estimation of success in comparison to untreated control tanks	Feasibility study complete Decision can be made with operators if longer-term experiment is to be conducted

Project activity and duration

Table 12 presents a model design for the activities of a projected barge experiment. This is only a model and any and all aspects of this preliminary study could be modified. Phase I includes the preliminary planning and laboratory phases. Because the experiment may be designed around a potential Arctic application, many decisions would have to be made during this period. The major experimental objective would be to determine whether seeding with microbial consortia is essential, or whether the indigenous microbial populations can carry out the biodegradation effectively. It would be important, therefore, to establish baseline characteristics of oil and microbes. The planning sessions would determine such parameters as time frame, appropriate season, how many tanks,[15] etc. Perhaps the most important decision in this first phase is whether this experiment is to be a "best-case" scenario. This would determine the choice of oil or sludge in terms of age, weathering, gravity, degree of wax, asphaltenic composition, etc.

During Phase II of the study, much of the engineering and retrofitting would be accomplished. Steam lines normally used for heating oil in the winter would be used to establish temperature control within the experimental tanks. Air supply systems would have to be investigated to determine whether the compressor facilities on board are suitable. Moreover, a small laboratory would have to be outfitted, and decisions made regarding its level of sophistication.

The actual experiment would be conducted during Phase III. Samples would be collected and on-board analyses made to follow growth of the organisms, emulsification of the sludge, and physical disappearance of the oil. In addition, samples would be collected and stored appropriately for subsequent chemical analysis. The duration of the experiment would depend to some extent on the venue of the barge. If the experiment is conducted in Arctic waters, there may be several weeks during which the bioremediation can take place. Under those conditions the engineers and microbiologists may want to examine the cycling of a portion of the contents of one tank as an inoculum for the second. During this phase of the project, selected isolation of organisms with specialized capabilities could also be attempted (PAH degraders, VOC degraders, sulphate-reducers, aromatic oxidizers etc.).

Phase IV would include post-experimental evaluation of the test results, examination of the tanks themselves, monitoring of the head-space for explosive hydrocarbons (were

[15] *For each variable, two tanks are required: one is treated and the other is an untreated control.*

the tanks in fact gas-free?), and preparation of a coordinated report. The overall project is expected to last about two years, depending on the time taken to get approval for use of the barge, etc.

Anticipated costs

Each of the two technical contractors, i.e., the microbiologist and the engineer, are expected to cost in the order of $60 000 a year. This should cover the cost of any assistance (each is likely to require one full-time assistant), consulting fees to each of the contractors, laboratory supplies, small-scale equipment, and overhead. It would not cover the cost of sophisticated chemical analysis (which can be done through a commercial laboratory) or of retrofitting costs or large-scale purchases of nutrients for the experiment itself. The cost of the voyage, use of the ship, food for the technical staff, etc., should be determined by the TDC coordinator.

7.1.2 Tankers as bioreactors for *in situ* biodegradation of oil and sludge

Major objectives

To confirm the feasibility of using tanks on board oil-tankers as *in situ* bioreactors for the bioremediation of hydrocarbon wastes such as sludges, bottom sediments, grease, and residual oil.

Anticipated benefits

The results from this R&D program are likely to lead to the development of a new bioremediation technology for cleaning and degassing of slop tanks, ballast tanks, and cargo tanks on oil tankers, barges, and other oil-transporting vessels. In addition to bringing about maximum biodegradation of the pollutants, the technique is likely to be cost-effective, scalable, and not labour intensive. The technology should also point the way to using on-board *in situ* bioremediation for the treatment of other pollutants, such as chemicals and xenobiotics.

Suggested participants

It is suggested that the project be managed by TDC with the cooperation of a microbiologist, a chemical/biochemical engineer, and a coordinator from the ship's crew (chief mate or chief engineer, possibly). To obtain the use of a vessel, the TDC manager would probably have to organize part of the project directly with an operator or with the help of a coordinating body or group.

Project activity and duration

Table 13 illustrates a plan for the activities of a projected tanker experiment. The overall program is designed for a two-year study culminating with an experiment on board. The experiment is divided into four overlapping phases. Phase I would include the laboratory and initial planning phases, with the major experimental objective of determining whether seeding is necessary for optimum biodegradation. The laboratory phase should also cover chemical analysis to assess degradation efficacy. In addition, during this phase, the vessel and type of tank should be chosen and the sludge samples given to the microbiologist. During the early part of Phase II the aeration and temperature control equipment would be designed, constructed, and installed. Possibly the steam heating facility normally used for heating oil in winter could be adapted to control the temperature. The actual tanker experiment itself would be conducted during Phase III. Samples would be collected and on board analyses made to follow growth of the organisms, emulsification of the sludge, and physical disappearance of the oil. Samples would also be collected for chemical analysis once the ship comes into port. In addition, the samples would be plated for specific oil-degrading micro-organisms that may be of interest for other projects (VOC degraders, PAH-degraders, corrosion-inducing organisms, etc.). The overall experiment should take less than the allotted month. Phase IV would include the post-experimental evaluation of the test results, examination of the tanks themselves, monitoring of the head-space for explosive hydrocarbons (were the tanks in fact gas-free?), and preparation of a coordinated report. The overall project is expected to last about two years, depending on the time taken to get approval for use of the tanker, etc.

Anticipated costs

Each of the two technical contractors, i.e., the microbiologist and the engineer, are expected to cost in the order of $50-$60 000 a year. This should cover the cost of any assistance (each is likely to require one full-time assistant), consulting fees to each of the contractors, supplies, laboratory analysis, and overhead. It would not cover the costs of equipment construction (the aeration equipment should cost between $2500 and $5000 for polypropylene piping and the labour to construct the aeration network, drill the air holes, etc.). The equipment for temperature control would have to be determined by the end of Phase I. The cost of the voyage, use of the ship, food for the technical staff, etc., should be determined by the TDC coordinator.

Table 13 – Bioremediation aboard an oil tanker

	PHASE I	PHASE II	PHASE III	PHASE IV
SHIP COORDINATOR AND ENGINEER	Choose vessel Provide sludge samples Determine which tanks to use and begin planning for tanker experiment	Complete planning schedule for experiment Construct and install aeration system and temperature control system (if necessary) Stockpile and store nutrients for experiment	Conduct experiment	Obtain final microbiological analyses and carry out final analysis
MICROBIOLOGIST	Laboratory experiments Test existing consortia and isolate new consortia to determine requirement for seeding Analyze residues in all cases to determine extent of biodegradation Together with bio-chemical engineer determine optimal parameters	If necessary begin large-scale preparation of microbial consortia as inocula	Monitor growth and emulsification during experiment Monitor oil degradation by disappearance Plate for specific organisms Collect samples for chemical analysis of residual hydrocarbons	Prepare final report
BENCHMARKS	Decision as to whether seeding necessary Parameters for bio-degradation in place Vessel chosen Tanks chosen (slop tanks, ballast tanks, etc.) Preliminary working schedule and time-table established	Tanks retrofitted If necessary, inocula prepared	Preliminary data on success of experiment Estimate of rate and extent of degradation Preliminary data on microbial growth and comparison with untreated tanks	Feasibility study complete Decision on whether another experiment should be conducted can be made with operators

7.1.3 Ships as bioreactors for *in situ* bioremediation of oil and grease in bilges

Major objectives

To test the feasibility of using *in situ* bioremediation aboard ships for the treatment of hydrocarbon pollution in bilges.

Anticipated benefits

The ability to carry out bioremediation of greases and oils in bilges offers the possibility of overcoming the spill problem arising from the indiscriminate and illegal pumping of bilges. In the long term, the technology and results stemming from this R&D program could lead to the development of a dedicated vessel, converted to a bioreactor facility, which would circulate in a specific area, collect bilges from ships, and carry out the bioremediation.

Suggested participants

Since the project follows naturally from the tanker program outlined in 7.1.1, the same participants, Dr. Donald Westlake and Dr. Ronald Neufeld, could be involved, along with the ship coordinator and the TDC manager.

Project activity and duration

The bilge bioremediation project would involve many of the same activities anticipated for the tanker project and could be carried out simultaneously by the same group. No additional engineering would be required. However, the microbiology group would be involved in isolating and testing specific consortia for their effectiveness with a different hydrocarbon substrate, and many of the parameters for biodegradation during the laboratory period could be different. Thus Phase I of the project might involve an additional microbiology assistant. Phase II of the bilge program could also be performed simultaneously with the ship experiment described in 7.1.1. In this case, two small tanks could be used for the bioremediation of the tanker bilges pumped into the tank for this purpose. The design of this experiment would depend on the parameters and guidelines obtained during Phase I. Since the questions for the bilge experiment are similar to those for the tanker experiment, the sampling schedules and retrofitting could be done at the same time. Since in the long term the bioremediation vessel is likely to receive bilges from other ships regularly, an important parameter to be investigated during Phase II would be the overall turnover time of a remediating cycle. The decision might be taken at the end of Phase I, therefore, to use more than one experimental tank, either concurrently or sequentially.

Anticipated costs

The costs would be considerably lower if the bilge and sludge experiments could be done during the same voyage. Under these conditions, the cost of an additional microbiology assistant would be about $30 000 a year. Additional piping and labour for the installation of air hoses is likely to cost an additional $2000. If this experiment is to be done alone, then the costs are likely to be similar to those described in 7.1.1.

7.1.4 Bioremediation of deicing fluids

Major objectives

To extend the technology for the bioremediation of deicing fluids to include the remediation of newer polymeric materials at lower temperatures.

Project background

Deicing fluids used in northern airports include Type I fluids, which are used in high volume and applied at high temperature, and Type II fluids, which are used in lower volume and contain a low concentration of a polymeric thickening agent that permits the formation of a coating on the surface of the metal. Type II fluids are expected to be used increasingly as airports become more congested, and as planes wait longer on the runway for takeoff they will need more deicing fluid. While Type I fluids contain minor amounts of diethylene glycols (DEG), Type II fluids usually contain much higher concentrations. Type II fluids cannot be recycled and may be more difficult to biodograde, particularly at lower temperatures. This project would study a number of parameters of significance in the bioremediation of a variety of these newer deicing materials.

Project activity and duration

The project would involve the isolation and characterization of a number of glycol degrading organisms with an emphasis on those that can degrade the diethylene derivatives. Organisms would be isolated from specific low temperature environments on the basis of their ability to utilize diethylene glycol (as a model substrate) as a source of carbon and energy. Effects of temperature and glycol sensitivity would be determined along with the potential for utilizing the urea already present on the runway as a source of nitrogen. It is recommended that the project be carried out for a first phase of about a year to compare existing strains (if available) with those isolated during the project. On the basis of the

progress during Phase I the decision could be made to proceed to a second phase that would include determination and optimization of performance characteristics, scale-up into large-scale reactors, etc.

Suggested participants

The project would be managed by TDC but probably should include the participation of the Transport Canada team headed by Youssef Sabeh, already in place in Dorval. Since this group works primarily on activated sludge systems it would be useful to include a microbiologist in the project. One group already involved in specific strain isolation and characterization is Microtek Research & Development Ltd. In addition, the author has learned that Dr. McCready of C.A.R.E. International in Calgary is continuing his R&D projects on bioremediation of deicing fluids.

Anticipated costs

A one-year study by this group, including the isolation of the appropriate strains, could cost in the order of $30 000.

7.1.3 Biological basis of corrosion in ships

Major objectives

To determine the relative contribution of microbial activity to the corrosion of the outside surface of ships' hulls.

Project background

As pointed out in Section 4.3, corrosion of steel structures represents a major economic loss to the shipping industry. Bacteria contribute heavily to this process and are thought to bring about corrosion in a variety of ways. Two of the major sources of bacteria-mediated corrosion in buried pipelines and cables are the sulphate-reducing (SRB) and acid-producing (APB) populations, both of which are very heterogeneous. In this project a survey would be conducted to monitor the heterogeneity and diversity of microbial populations and any fluctuations that occur during the process of corrosion.

Anticipated benefits

Prevention of biological corrosion will require a detailed knowledge of the organisms involved, since new design criteria for corrosion inhibitors, biocides, and appropriate alloys will depend on the specific microbial processes likely to occur. As a first step in unravelling the corrosive environment, this project would allow an estimation of the actual contribution of microbes to this process.

7.2 Potential projects in the long term

7.2.1 Personnel requirements

The projects recommended in Section 7.1 could most likely be managed by personnel already working in TDC, because the objectives of the projects require that the biological process bring about a specific effect; e.g., the microbial process either works to clean out the tanker or it doesn't. In the long run, biologically oriented projects probably will need to be managed by personnel with a more microbiological or biotechnological orientation. Such an individual would assume responsibility for all the biotechnologically oriented projects related to marine oil spills and allied technologies. The person chosen should have a Masters degree in chemical or biochemical engineering with some academic or professional experience in biotechnology. This would allow the candidate to work on additional projects that may be of interest to TDC in the future (fuel upgrading, emulsion-based fuels, corrosion detection and prevention, VOC treatment, etc.). The Chemical Engineering Department at McGill University has a special program in Biochemical Engineering run by Profs. Neufeld, Volesky, and Cooper. Their graduates are ideally suited for the TDC position. Similarly, the Microbiology Department at UBC also has a very good training program in environmental microbiology. The University of Waterloo Centre, under the direction of Dr. Murray Moo-Young, remains one of the major Canadian centres for Biochemical Engineering. It is understood that the need for such an individual is predicated on the degree to which TDC can identify a role for itself in the whole area of Environmental Biotechnology and Transportation.

7.2.2 Participation in a long-range field experiment

It is commonly accepted that bioremediation of marine oil spills must undergo many field trials under controlled conditions to gain public acceptance as a useful and "safe" technology. The field experiments involve intentional spilling of oils onto preselected terrestrial environments and monitoring of the results of various clean-up methods.

including bioremediation. These long-term experiments require intensive planning and permission must be obtained from a host of regulatory agencies. Such planning usually takes from three to five years, and involves the participation of a number of government agencies, the Coast Guard, and a variety of consultants and subcontractors. Moreover, since the U.S. still does not allow such spills, the EPA and other U.S. groups generally assume part of the support for the trial in exchange for the right to carry out some of their own experimentation. Because current opinion among the experts is that bioremediation is not practical in the open sea, the work has been restricted to coastal areas. However, there may be an advantage to treating polluted waters with suitable encapsulated consortia that can stick to the oil, that are adapted to begin biodegradation without a lag, and that carry their own nutrients, so the whole package can be sprayed from an aircraft or from a helicopter.

Initial laboratory phases of the project would include developing the encapsulation technology and trying some form of entrapment (such as use of the oleophilic peat). The experiment would probably be carried out initially in small tanks in which wave action could be simulated, but the experimental system would still permit monitoring of the biodegradation.

A more difficult problem is to conduct a larger-scale experiment; sampling from the open sea would be difficult. One potential approach is to carry out the experiment in a calmer inlet in which the oil reaching the beaches after treatment could be continuously monitored.

TDC management would probably be restricted to this aspect of an overall field experiment and would depend on a high level of coordination with other government agencies such as Environment Canada, Fisheries and Oceans, EPA, and CPA.

7.3 Towards an integrated program of biotechnology and bioremediation at TDC

7.3.1 Scope and range of activities

One of the author's major objectives during his assignment at TDC under the Visiting Experts Program was to highlight a series of programs in which TDC could make original contributions to the development of specific technologies in the area of bioremediation of marine oil pollution. During his stay, the author decided to expand the scope of the survey to examine a number of additional biotechnologies that might significantly affect

the transportation industry. The *in situ* bioremediation projects involving the conversion of ocean-going vessels into mobile bioreactors should receive the highest priority for the following reasons:

- To the best of the author's knowledge programs such as these are not being carried out in Canada. Any successful technological development is likely to be "state of the art".
- The projects lend themselves to cooperation with other government agencies that can benefit from the R&D (e.g., the Coast Guard, Indian and Northern Affairs, and Industry, Science and Technology).
- The expertise that can emerge along with the technological development may enable TDC to become involved in other transport-related projects in biotechnology.
- At various stages of the program, patentable discoveries, prototypes, and other forms of intellectual property are likely to be forthcoming.

In the next section a preliminary scheme for the entrance of TDC into an integrated R&D program is outlined.

7.3.2 An integrated program for transport-related biotechnology at TDC

The scheme is outlined in Table 14. It is not recommended that TDC become immediately involved in a large-scale commitment. Rather, it is suggested that during a first phase of two or three years one or more short-term experiments be undertaken. Some of these, shown in Table 14 in order of priority, have been discussed in the sections above. The enthusiasm of some senior management in the Coast Guard for the concept of the barge as a mobile bioreactor should be taken into account during the initial planning. This first phase may require additional time to allow the evaluation of progress during the second phase. By this time a significant data base of results and acquired experience will have been established, permitting the rational evaluation of the progress, potential, and level of commitment required to embark on the longer-term projects in the third phase. In the author's opinion, the transition from Phase II to Phase III will require at least two conditions to be met: that a full-time staff officer be hired to handle the bioremediation and biotechnology program; and that questions in Phase II, such as those outlined in Table 14, can be clearly answered.

Table 14 – Bioremediation at TDC

PHASE I	PHASE II	PHASE III
▪ *IN SITU* EXPERIMENTAL BARGE ⬇ PLAN, COORDINATE, EXECUTE EXPERIMENT ▪ *IN SITU* EXPERIMENTAL TANKER ⬇ PLAN, COORDINATE, EXECUTE EXPERIMENT ▪ *IN SITU* EXPERIMENTAL BILGE CLEANING ⬇ CONCURRENT WITH TANKER EXPERIMENT ▪ DEICING FLUIDS ⬇ LOW TEMPERATURE TYPE II FLUIDS ▪ BIOCORROSION OF SHIP HULLS ⬇ DIAGNOSTIC PROGRAM ESTIMATE EXTENT ▪ HEAVY OIL DESULPHURIZATION ⬇ MICROBIAL REMOVAL OF ORGANIC SULPHUR	▪ EVALUATION ⬇ DOES IT WORK? WORTH PURSUING? AT WHAT COST? WITH WHOM? INCREASED STAFF?	▪ LARGER-SCALE BARGE EXPERIMENT ▪ LARGER-SCALE TANKER EXPERIMENT ▪ DESIGN OF BIOREMEDIATION BARGE TO BEGIN ▪ SCALE-UP OF DEICING BIOREMEDIATION ▪ INVESTIGATION OF DESIGN OF OTHER MOBILE BIOREACTORS ▪ PARTICIPATION IN LARGE-SCALE FIELD TRIAL

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APPENDIX A

TDC Workshop: Program and Speakers

WORKSHOP ON BIOREMEDIATION OF MARINE OIL POLLUTION

HÔTEL LE MARITIME
Beaupré Room
(Mezzanine)

PROGRAM

MORNING SESSION
Beaupré Room

9:00 - 9:10	**Introduction**	Dr. David Gutnick, Chairman
9:10 - 9:40	**Lecture I: Microbiology**	Dr. Donald Westlake
9:40 - 10:20	**Keynote Address**	Dr. Ronald Atlas
10:20 - 10:45	*Coffee break*	
10:45 - 11:15	**Lecture II: Field Experiments**	Dr. Ken Lee
11:15 - 11:45	**Lecture III: The Arctic System**	Mr. George McCormick
11:45 - 12:15	**Lecture IV: Allied Technologies**	Dr. Tom Jack
12:15 - 1:30	*Lunch*	

1:30 - 3:30 **CONCURRENT SESSIONS**

Group 1: Microbiology *Beaupré 1*	Dr. Charles Greer
Group 2: Field Experiments *Beaupré 2*	Mr. Mervin Fingas
Group 3: Allied Technologies *Beaupré 3*	Dr. Ronald Neufeld

AFTERNOON SESSION
Beaupré Room

3:30 - 4:00	*Coffee break*	
4:00 - 5:00	**Summary and General Discussion**	Dr. David Gutnick

ATELIER SUR LES BIOTECHNOLOGIES ET LA POLLUTION DES MERS PAR LES HYDROCARBURES

HÔTEL LE MARITIME
Salon Beaupré
(Mezzanine)

PROGRAMME

9h00 à 9h10	**Introduction**	David Gutnick, Président
9h10 à 9h40	**Conférence I : Microbiologie**	Donald Westlake
9h40 à 10h20	**Discours – Programme**	Ronald Atlas
10h20 à 10h45	*Pause*	
10h45 à 11h15	**Conférence II : Expériences pratiques**	Ken Lee
11h15 à 11h45	**Conférence III : Écosystème arctique**	George McCormick
11h45 à 12h15	**Conférence IV : Technologies connexes**	Tom Jack
12h15 à 13h30	Dîner	
13h30 à 15h30	**ATELIERS PARALLÈLES**	
	Groupe 1 : Microbiologie *Beaupré 1*	Charles Greer
	Groupe 2 : Expériences pratiques *Beaupré 2*	Mervin Fingas
	Groupe 3 : Technologies connexes *Beaupré 3*	Ronald Neufeld
15h30 à 16h00	*Pause*	
16h00 à 17h00	**Récapitulation et débat**	David Gutnick

SPEAKERS AND CHAIRMEN FOR THE TDC WORKSHOP ON BIOREMEDIATION OF MARINE OIL POLLUTION

ORGANIZER AND CHAIRMAN

David Gutnick — Professor of Microbiology and Biotechnology, Tel Aviv University; and Visiting Expert Environmental Biotechnology, TDC, Montreal

KEYNOTE LECTURE

Ronald Atlas — Professor of Microbiology, University of Louisville, Kentucky

MORNING PRESENTATIONS

Donald Westlake — Westec Microbes Ltd., and Professor of Microbiology, University of Alberta, Edmonton

Kenneth Lee — Research Scientist, Maurice Lamontagne Institute, Mont-Joli, Quebec

George McCormick — Senior Environmental Officer, Northern Oil and Gas Directorate, Indian and Northern Affairs, Hull

Thomas Jack — Manager, Applied Sciences, NOVA HUSKY Research Corporation, Calgary

SESSION CHAIRMEN

Charles Greer — Research Officer, Environmental Engineering Group, Biotechnology Research Institute, National Research Council Canada, Montreal

Mervin Fingas — Chief, Emergencies Science Division, Technology Development Branch, River Road Environmental Technology Centre, Ottawa

Gary Sergy — Manager, Western Office, Technology Development Branch, Environment Canada, Edmonton

Ronald Neufeld — Associate Professor of Chemical Engineering and Director of McGill Centre for Bioprocess Engineering, McGill University, Montreal

Dr. David Gutnick

Dr. David Gutnick is Professor of Microbiology and Biotechnology at Tel Aviv University. He received his MSc from the University of California in Food Science and Technology and his PhD in Microbiology from the University of Southern California in Los Angeles. Before moving to Israel in 1969, he spent two years in the Laboratory of Molecular Biology at the U.S. National Institute of Health (NIH).

Specializing in bioremediation of oil pollution, David is an international leader in Petroleum Microbiology and senior author of the first patents for the use of bacteria to clean oil storage containers in ocean-going vessels and on land. When these patents were acquired by a U.S. company, he became a pioneer in the area of technology transfer from laboratory to industry.

He has been a Visiting Professor at the University of Chicago, MIT, Roche Institute of Molecular Biology, NIH, University of Amsterdam, Mexican Institute of Genetic Engineering and Biotechnology, Chinese Academy of Sciences in Beijing, and the National University of Singapore. He has also served on international biotechnology panels and committees, including the UNIDO planning committee for the Institute of Biotechnology in Trieste, Italy.

While at TDC, David will prepare a comprehensive assessment of the potential application of bioremediation and biodegradation measures to combat the effects of marine oil spills. He also intends to identify the available research and development capabilities throughout Canada, and to recommend avenues of research.

Dr. Ronald Atlas

Dr. Ronald Atlas is Professor of Biology at the University of Louisville, and since 1988 has served as Associate Dean of Arts and Sciences. He received his MSc and PhD in Microbiology at Rutgers the State University.

Recognized as one of the world's pre-eminent microbiologists working in the area of Petroleum Microbiology, Bioremediation and Environmental Biotechnology, Ron has been a pioneer in the biodegradation of petroleum in low temperature environments. As early as 1973, Atlas and Bartha pioneered the use of hydrophobic fertilizers for stimulating the biodegradation of oil slicks. This approach formed the basis of the *Exxon-Valdez* bioremediation experiment in Alaska. Ron served on the EPA committee overseeing the design and implementation of this effort, and has been associated with the *Exxon-Valdez* Environmental Assessment Program. In addition, he has served on the Recombinant Advisory Committee (RAC) of the NIH and is a member of the Environmental Committee of the Public Affairs Board of the American Society of Microbiology.

Dr. Atlas serves on the editorial boards of several international journals including *Applied and Environmental Microbiology*, *Advances in Microbial Ecology*, the *Journal of Industrial Microbiology*, and *Bioscience*. He is the author and editor of a number of key books including *Petroleum Microbiology*, *Microbiology: Fundamentals and Applications*, *Experimental Microbiology: Fundamentals and Applications*, and *Microbial Ecology: Fundamentals and Applications*. Ron received the University of Louisville Research Excellence Award in 1985, and this year received The American Society of Microbiology Award in Applied and Environmental Microbiology.

Dr. Donald Westlake

Don is President of Westec Microbes and Professor Emeritus at the Department of Microbiology, University of Alberta, where he still holds a one-quarter time appointment as Professor. He is also a Visiting Professor of Microbiology at the University of Victoria. As Professor and Chairman of the Department of Microbiology at Edmonton between 1969-1989, he succeeded in building one of the most important research centres for environmental microbiology in Canada.

With over 20 years experience in hydrocarbon and environmental microbiology, he has concentrated his research efforts on establishing the physical-chemical changes in oils brought about by microbial activity and on understanding the conditions controlling the biodegradation of oil *in situ* (i.e., in reservoirs or oil spilled in the environment). His work has culminated in over 60 publications in various research journals and in numerous invited presentations all over the world. His research efforts have earned him an international reputation on interactions of oil and microbes as well as on technology transfer in the application of biotechnology to clean-up of oil and related spills.

Professor Westlake has been Chairman and served as a member of numerous NSERC Committees, including the Cell Biology and Genetics Grants Committee and the Advisory Committee on University Industrial Grants. In addition, he served on the U.S. Department of Energy Fossil Energy Advanced Research Projects Assessment Committee, chairing the Panel on Biotechnology of Coal Conversion, and is a member of the EPA Advisory Committee on Bioremediation and the *Valdez* Oil Spill.

Don was editor of the *Canadian Journal of Microbiology* from 1984-1989 and is also on the editorial board of *Geomicrobiology*. In 1985 he received the Canadian Society of Microbiologists Award as well as a McCalla Professorship at the University of Alberta, and in 1986 he was awarded the University of Alberta Research Prize.

Dr. Kenneth Lee

Kenneth Lee received his doctorate in microbial ecology from the University of Toronto. He was an NSERC Visiting Scientist at the Ocean Chemistry Division, Institute of Ocean Sciences, British Columbia, where he was involved in a multi-disciplinary program using SEAFLUX enclosures (mesocosms) to evaluate the role of micro-organisms in the biodegradation of crude oil and oil dispersants in pelagic ecosystems.

As a research scientist under contract at the Bedford Institute of Oceanography, Dr. Lee conducted a multi-year project (funded by the Office of Energy Research and Development), to evaluate the feasibility of enhancing the biodegradation of crude oils and condensates stranded or buried on beach and salt-marsh environments on the Atlantic coast of Canada. In addition, he was the senior biologist on several research expeditions studying natural submarine oil gas seeps in the Canadian Arctic.

In 1989, Dr. Lee joined the Department of Fisheries and Oceans as a research scientist with the Chemical Oceanography and Contaminants Division, Maurice Lamontagne Institute, Mont-Joli, Québec. His current projects on hydrocarbons include:

- the development of nutrient-enhanced bioremediation technologies for the treatment of oil spills;
- experiments to investigate the possibility that the nutritional needs of benthic organisms may be significantly supplemented natural hydrocarbon seepages;
- the development of microbial assays to assess the impact of offshore drilling operations on benthic biota; and
- studies on the biodegradation of polyaromatic hydrocarbons in the Saguenay Fjord.

Mr. George McCormick

George has been a Senior Environmental Officer with the Northern Oil and Gas Lands Administration and has recently joined Indian and Northern Affairs Canada. He has been involved in work on containment and recovery of oil spills since 1980.

He currently coordinates the environmental review process for drilling program approvals with other federal and territorial agencies and the oil and gas industry, to ensure compliance with environmental legislation.

Among his many activities, George has:

- conducted seminars for industry and government regarding spill clean-up and land reclamation;
- supervised contractors for the containment and recovery of oil and salt spills, including reporting to landowners, the public, and regulatory agencies;
- initiated and devised cost-effective reclamation programs to restore approximately 35 landsites per year – sites affected by erosion, or soil sterilant, oil, or salt spills; and
- inspected oil and gas and geological exploration programs in the Arctic Islands to ensure compliance with the Territorial Land Use Regulations.

George is well acquainted with all aspects of oil exploration and drilling programs in the Arctic.

Dr. Thomas Jack

Tom, a PhD in Organometallic Chemistry and Catalysis, is Manager of Applied Sciences at NOVA HUSKY Research Corporation, with responsibility for research programs in enhanced oil recovery, biosciences, and corrosion. He has been with NOVA HUSKY since 1981. He has a background in R&D areas as diverse as:

- microbially enhanced oil production through the *in situ* use of bacteria in the oil reservoir itself;
- the role and control of bacterial corrosion;
- on-site waste degradation;
- sulphate-reducing bacterial activity in an Arctic lake, Little Cornwallis Island, N.W.T.;
- petroleum microbiology, with special emphasis on the recovery and modification of oil through microbial action, biofouling of fuel storage and delivery systems, and microbial degradation problems in ships' crankcase oils and machine shop cutting oils;
- the chemically and biologically assisted leaching of metals from Athabasca oil sand cokes;
- the toxicity of metal leachates by static bioassay;
- the immobilization of whole microbial cells for the enzymatic conversion of chemicals in a continuous flow reactor; and
- the production of lipases from *Candida lipolytica*.

Dr. Charles Greer

Charles, who earned his PhD in Microbiology at McGill University, is Research Officer in the Environmental Engineering group at the Biotechnology Research Institute, National Research Council of Canada. He is currently working on the following:

- development and application of catabolic gene probes for microbial characterization and monitoring of soil and water contaminated with chlorinated and non-chlorinated organic (aromatic) compounds;
- development of reporter gene systems to monitor the fate of bacteria introduced into pristine and contaminated soil and water;
- enhancement of *in situ* bioremediation; and
- biotreatability test protocol development for soils contaminated with petroleum hydrocarbons.

Mr. Mervin Fingas

Merv Fingas is Chief of the Emergencies Science Division, Environment Canada. This division is responsible for performing research on oil and chemical spill behaviour and control. Mr. Fingas's current responsibilities include the management and performance of research related to the following areas:

- fate, behaviour, and modelling of oil and chemical spills generally;
- development of analytical and remote sensing techniques;
- preparation of spill manuals, information compilations, and overview reports; and
- testing and development of chemical spill treating agents.

Mr. Fingas has three master's degrees: one in chemistry, one in business, and another in mathematics. He also has technical credentials in machining and electronics. He has devoted the last 17 years of his life to spill research and has over 150 papers and publications in the field.

Dr. Ronald J. Neufeld

Ron Neufeld received his B.Sc.Agr. (Microbiology) in 1973 from Macdonald College of McGill University. He was then employed by Domtar, where he worked in the Engineering and Process Development Group on Environmental Impact Assessment related to gaseous and liquid discharges from plants involved in the production of pulp and paper, chemicals, and construction materials. Following this industrial experience, Ron went to the University of Western Ontario, where he completed a PhD in Chemical and Biochemical Engineering. Ron has been in the Department of Chemical Engineering at McGill University since 1980, and is presently Associate Professor of Chemical Engineering and Director of the McGill Centre for Bioprocess Engineering. Ron's research interests are in fermentation and enzyme process development; immobilization of micro-organisms, enzymes, and biologically active materials (DNA, oxygen carriers, pigments, pharmaceuticals); and the development of microencapsulation technologies in the biotechnology and environmental fields. He recently returned from a sabbatical year serving as Visiting Professor at the Technion (Haifa) and the Hebrew University of Jerusalem.

APPENDIX B

List of Workshop Participants

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